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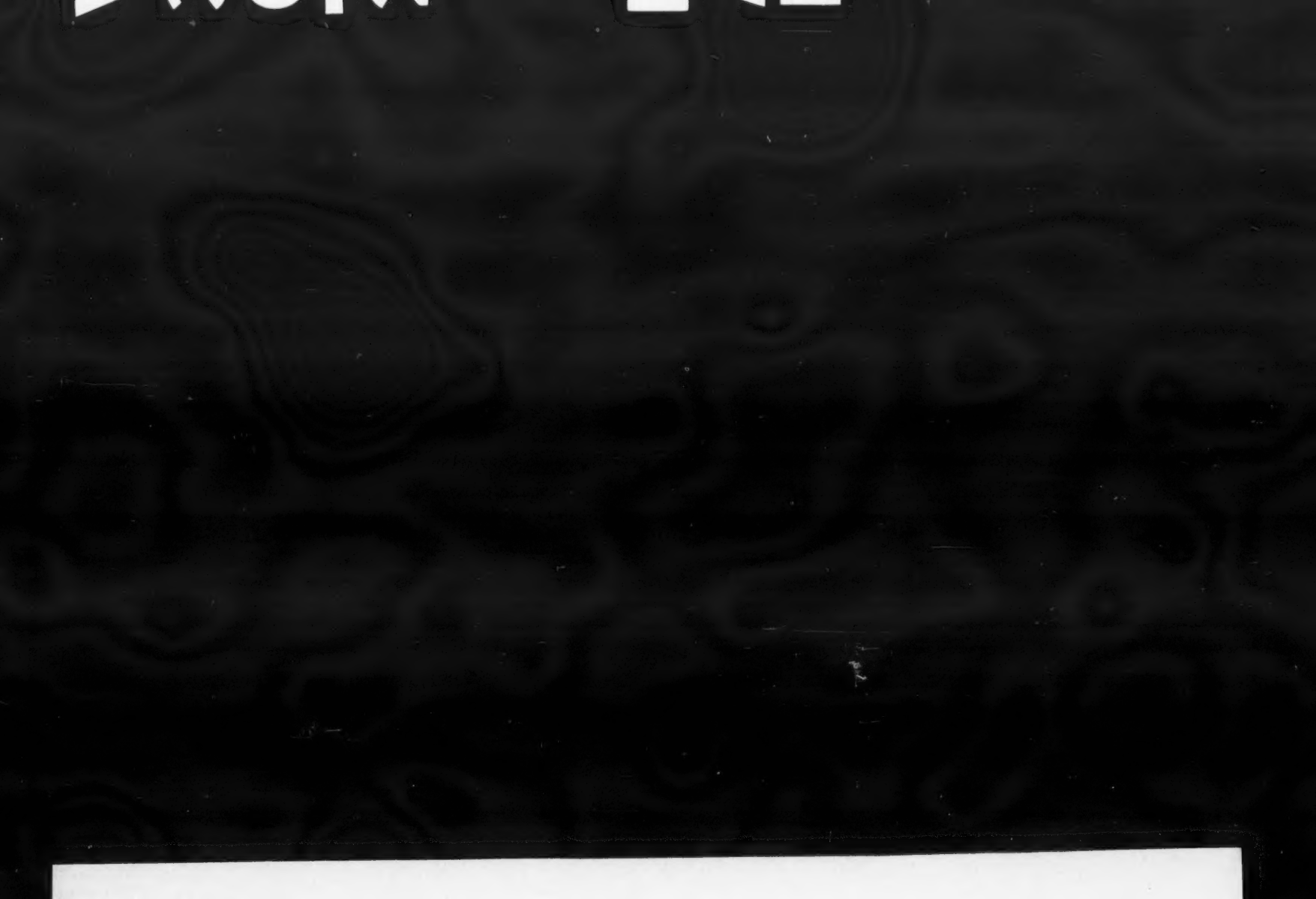

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MINERAL NUTRIENT CONTENT OF SOME FOREST PLANT LEAVES AND OF THE HUMUS LAYER AS RELATED TO SITE QUALITY[1]

D. GAGNON,[2] A. LAFOND,[3] AND L. P. AMIOT[3]

Abstract

The nutrient content of the humus and of some of the plants characteristic of different site classes has been studied. Analyses carried out on fresh healthy leaf material from typical forest plants indicated no differences within a given species that could be related to site quality. Available nutrient content of the A_0 horizon collected in the same environment is, however, closely related to the productivity of the sites. It seems, therefore, that the differential accumulation of nutrient elements in the cells of the forest plants studied depends upon the inherent properties of the species rather than on the quantity of available nutrients in the humus on which they grow.

Introduction

The purpose of this study was to determine the relationship that might exist between the mineral content of the healthy leaves of certain lesser forest plants and the quantity of available nutrients in the humus in stands of various productivity.

A lack of emphasis on research of this nature has been observed by Kozlowski (13), and most of the few studies dealing with extraction of mineral nutrients from lesser vegetation foliage do not consider the possible correlation between forest site quality and mineral nutrient content of leaf tissues; moreover, very few attempts have been made to correlate the results with site quality. However, a number of references are made to investigations dealing with tree foliage, and the results concerning the relationship between the nutrient status of trees and that of the site are apparently conflicting.

According to Mitchell (20), whose opinion is widely shared by forest ecologists, the nutrient content of plant tissues growing on a site could give a reliable index of quality. However, Hoagland (12), Lundegarth (17), and others have shown that plants can accumulate in the vacuoles of their cells mineral nutrients to a concentration much greater than that of the external

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Contribution from the Forest Research Division, Department of Northern Affairs and National Resources, Valcartier Forest Experiment Station, Quebec, and the Faculty of Forest Engineering, Laval University, Quebec.

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environment (in this case, the humus layer). Aaltonen (1) and Russell (23) have found that the quantity of ions accumulated in plant tissues varies from one species to another.

In view of such conflicting results in the literature the analyses of lesser vegetation would appear to be necessary for a more complete appreciation of the relationships between the mineral content of leaves in some forest plant indicators and that of decomposed detritus.

Material and Methods

Field Procedure

Samples of plant tissues and the humus layer were collected by two field parties over a 2-week period during the latter part of August 1955 (after the growing season) in three widely separated parts of the Northeastern Coniferous Section (B.1) of the boreal forest region (Halliday (9)). Samples were obtained for 11 site-types differing in their ecological structure and representing four site classes as described by Linteau (16) and named: *Dryopteris–Oxalis* (site class I), *Hylocomium–Oxalis*, *Hylocomium–Cornus*, *Hypnum–Cornus*, and *Hypnum* (site class II), *Sphagnum–Cornus*, *Kalmia–Vaccinium*, and *Hypnum–Kalmia* (site class III), and *Sphagnum–Ledum* and *Rubus–Chamaedaphne* (site class IV). Although the brief period available for collection of samples did not permit every site-type to be found on all the sampling areas, each site-type was adequately represented. Only healthy leaf material, gathered over the entire surface of a sample plot, was used for analysis. Humus (H layer) samples from 10 different locations within a plot were mixed together for analysis.

Laboratory Procedure

Oxidation of the organic matter was accomplished by successive treatments with nitric acid and hydrogen peroxide. In order to release any ammonium salt that might have been formed during the digestion and to coagulate the silica contained in the samples, the mineral residue was treated with hydrochloric acid and heated to 400° C. for 30 minutes. It was then dissolved and diluted to 100 ml. in 0.6 *N* HCl.

The calcium and potassium content of the solutions was determined by the use of a Beckman spectrophotometer, model "D.U.", equipped with an oxyacetylene flame attachment. Magnesium determinations were repeated several times using thiazole yellow according to the procedure described by Drosdoff and Nearpass (6) and the flame spectrophotometry after Toth *et al.* (28) with the photomultiplier attachment. The manganese was determined colorimetrically by oxidation to permanganate using sodium periodate as recommended by Willard and Greathouse (32). The readings were made by means of a Coleman spectrophotometer (model 14). Iron was determined colorimetrically by the formation of the red orthophenanthroline – ferrous iron complex according to the method of Wenger and Duckert (31). Phosphorus was assumed to be present as orthophosphate. This was ascertained by the molybdenum blue reaction of Chapman (5), using stannous chloride

as the reducing agent. A 2 ml. aliquot of the solution was diluted to the point where phosphorus determination would follow the laws of Lambert and Beer.

Exchangeable ions in the A_0 horizon were extracted using a solution of ammonium acetate at pH 7.0 (24). The pH values were determined with a Beckman H2 potentiometer (glass electrodes).

As even the highest grades of reagents often contain traces of impurities which might interfere with the results, a blank was analyzed the same way as the soil or tissue samples and corrections made accordingly.

In order to ascertain the best estimates of individual cations, the calibration of the flame spectrophotometer (flame conditions, wave length setting, sensitive phototube, and slit width) was checked on a series of standards. Correction of the results obtained by flame photometric methods, reported by Spector (26) and Fieldes *et al.* (8) to be necessary owing to mutual cationic interference, was not made in the present study. The effects of the interference being asymptotic to a given value, the possible error in our results would in most cases be less than 5%.

From the 1649 individual determinations the average content of each mineral ion in every species in each site was calculated, along with the standard deviation and the standard error. After 33 determinations had been rejected because they differed from the average values by more than two standard deviations, new averages were calculated.

Results and Discussion

Results of the analysis are presented in three sections. The first one deals with the mineral ions found in the leaves of the forest plants studied, the second one with the data from humus analysis for each forest type where the tissues were collected, and in the third section the experimental evidence collected from the analysis is linked with the site values and the relative abundance of the plant species in the sample plot.

Plant Tissue Analysis

1. Calcium

Results of the calcium analysis are presented in Table I. The species analyzed can be divided into three groups according to their calcium content. The herbs appear to contain more calcium than the other plants here considered, but there is a marked difference between *Oxalis montana* and *Cornus canadensis* even though they are generally found over soils having about the same pH value. The shrubs, typical of the boreal forest, show a lower content of this cation than the herbs but markedly higher than the mosses. There is, however, no significant difference for the same species from different site-types.

One of the main roles of calcium within the plant and in the soil being the neutralization of excess acid, it may be expected that humus formed from a large amount of *Cornus* leaves, for example, will be less acid than that resulting

TABLE I

CALCIUM CONTENT OF PRINCIPAL PLANT INDICATORS IN DIFFERENT SITE CLASSES (IN P.P.M.)

Species	Site I	Site II	Site III	Site IV
Herbs				
Cornus canadensis L.	10,581	8,337	10,575	9,890
Oxalis montana Raf.	5,728	3,250	—	—
Dwarf shrubs				
Chiogenes hispidula (L.) T. and G.	3,450	3,120	2,890	2,952
Kalmia angustifolia L.	—	2,930	2,380	2,650
Ledum groenlandicum Oeder	—	2,470	2,400	2,080
Vaccinium pennsylvanicum Lam.	—	2,005	2,333	2,225
Mosses[a]				
Hypnum crista-castrensis Hedw.	1,750	1,605	1,242	1,577
Calliergonella Schreberi (Bry. Eur.) Grout	797	737	781	810
Sphagnum sp.	840	895	915	822
Hylocomium splendens (Hedw.) Bry. Eur.	770	780	675	894

[a]Throughout this paper the nomenclature of the mosses follows Conrad, Henry S. How to know the mosses. Wm. C. Brown Company, Dubuque, Iowa. 1944.

TABLE II

MAGNESIUM CONTENT OF PRINCIPAL PLANT INDICATORS IN DIFFERENT SITE CLASSES (IN P.P.M.)

Species	Site I	Site II	Site III	Site IV
Herbs				
Cornus canadensis L.	2,471	2,458	2,751	3,014
Oxalis montana Raf.	1,765	2,111	—	—
Dwarf shrubs				
Chiogenes hispidula (L.) T. and G.	1,930	1,926	1,810	2,124
Kalmia angustifolia L.	—	1,590	1,412	1,542
Ledum groenlandicum Oeder	—	1,097	1,625	1,256
Vaccinium pennsylvanicum Lam.	—	1,076	1,643	1,160
Mosses				
Hypnum crista-castrensis Hedw.	797	907	585	722
Calliergonella Schreberi (Bry. Eur.) Grout	467	499	483	513
Sphagnum sp.	777	605	548	626
Hylocomium splendens (Hedw.) Bry. Eur.	551	510	498	554

from a carpet of mosses. That this is indeed the case is shown in Table VIII where humus belonging to site class I is seen to have a pH value higher than humus from site class II or III.

2. Magnesium

The magnesium content (Table II) of the plant groups shows a tendency similar to that of calcium, although the differences between the herbs and shrubs are less marked. Magnesium is much lower in the mosses. There are no significant differences between the site classes considered.

3. Potassium

As a rule, potassium is absorbed by plants in larger amounts than any of the other mineral cations (12, 19). This holds true for the forest plants studied in this work (Table III). However, the potassium content differs from calcium and magnesium as far as plant groups are considered. The amount of potassium absorbed by the mosses is in the same concentration as in the *Oxalis montana* and *Cornus canadensis* leaves. It must be observed that *O. montana*, which has the largest amount of potassium in its tissues, reaches its optimum development only on the best sites. However shrubs, which are found more abundantly on the poorest sites, have less potassium in their tissues.

A rough examination of data from Table III would indicate that the potassium content in *Ledum groenlandicum* would vary according to the site quality. However, statistical analysis fails to support such conclusions.

TABLE III

POTASSIUM CONTENT OF PRINCIPAL PLANT INDICATORS IN DIFFERENT SITE CLASSES (IN P.P.M.)

Species	Site I	Site II	Site III	Site IV
Herbs				
Cornus canadensis L.	4,377	3,976	2,805	3,912
Oxalis montana Raf.	7,047	8,677	—	—
Dwarf shrubs				
Chiogenes hispidula (L.) T. and G.	2,490	2,645	2,360	3,010
Kalmia angustifolia L.	—	2,400	2,940	2,240
Ledum groenlandicum Oeder	—	1,380	2,080	2,170
Vaccinium pennsylvanicum Lam.	—	1,913	1,965	1,819
Mosses				
Hypnum crista-castrensis Hedw.	5,367	5,677	5,962	6,357
Calliergonella Schreberi (Bry. Eur.) Grout	4,410	5,167	4,823	4,650
Sphagnum sp.	6,550	4,963	4,816	5,074
Hylocomium splendens (Hedw.) Bry. Eur.	3,615	3,825	3,510	4,022

4. Iron and Manganese

Iron and manganese raise very difficult problems in nutrition studies; they are so similar in their physicochemical properties that ion antagonism is quite conceivable. However, they are also linked to very specific enzymatic properties of metabolism and thus they might react independently as suggested by Hewitt (10), Wallace (30), and McElroy and Nason (18).

It has been observed by Erkama (7) and Scott (25) that the manganese content of individual plants varies more than the content of other heavy metals, and this has been confirmed by our analysis (Table V). There does not seem to be any significant correlation between site value and the amount of this mineral in the leaves. In the mosses, however, more homogenous results were obtained than in other groups.

TABLE IV

IRON CONTENT OF PRINCIPAL PLANT INDICATORS IN DIFFERENT SITE CLASSES (IN P.P.M.)

Species	Site I	Site II	Site III	Site IV
Herbs				
Cornus canadensis L.	67	76	65	67
Oxalis montana Raf.	170	170	—	—
Dwarf shrubs				
Chiogenes hispidula (L.) T. and G.	75	98	73	75
Kalmia angustifolia L.	—	79	85	104
Ledum groenlandicum Oeder	—	113	77	111
Vaccinium pennsylvanicum Lam.	—	137	99	95
Mosses				
Hypnum crista-castrensis Hedw.	460	495	380	310
Calliergonella Schreberi (Bry. Eur.) Grout	433	774	417	392
Sphagnum sp.	338	455	340	386
Hylocomium splendens (Hedw.) Bry. Eur.	470	158	578	663

TABLE V

MANGANESE CONTENT OF PRINCIPAL PLANT INDICATORS IN DIFFERENT SITE CLASSES (IN P.P.M.)

Species	Site I	Site II	Site III	Site IV
Herbs				
Cornus canadensis L.	119	130	85	69
Oxalis montana Raf.	894	845	—	—
Dwarf shrubs				
Chiogenes hispidula (L.) T. and G.	1,970	2,123	1,777	2,300
Kalmia angustifolia L.	—	410	409	464
Ledum groenlandicum Oeder	—	366	409	315
Vaccinium pennsylvanicum Lam.	—	1,540	1,382	1,510
Mosses				
Hypnum crista-castrensis Hedw.	652	487	519	824
Calliergonella Schreberi (Bry. Eur.) Grout	554	489	487	467
Sphagnum sp.	385	444	361	463
Hylocomium splendens (Hedw.) Bry. Eur.	430	369	325	475

The iron content (Table IV) of the herbs and shrubs is generally much lower than the manganese content, while in the mosses the amounts of the two minerals are, with one exception, about the same: *Hylocomium splendens* on site II contains almost three times as much iron as manganese. This was true for 10 samples from different localities. It is hard at present to offer any explanation.

5. Phosphorus

Since phosphorus is highly mobile in plant tissues, a considerable variation of phosphorus content within a species can be expected. In Table VI the phosphorus content is presented for the species analyzed. As expected,

TABLE VI

PHOSPHORUS CONTENT OF PRINCIPAL PLANT INDICATORS IN DIFFERENT SITE CLASSES (IN P.P.M.)

Species	Site I	Site II	Site III	Site IV
Herbs				
Cornus canadensis L.	2,155	1,625	1,979	1,671
Oxalis montana Raf.	1,815	2,327	—	—
Dwarf shrubs				
Chiogenes hispidula (L.) T. and G.	1,390	1,347	1,240	1,238
Kalmia angustifolia L.	—	950	460	800
Ledum groenlandicum Oeder	—	1,755	1,675	1,830
Vaccinium pennsylvanicum Lam.	—	444	652	801
Mosses				
Hypnum crista-castrensis Hedw.	1,208	1,562	1,262	1,382
Calliergonella Schreberi (Bry. Eur.) Grout	890	998	1,149	837
Sphagnum sp.	1,487	1,538	1,093	1,395
Hylocomium splendens (Hedw.) Bry. Eur.	820	1,088	1,216	1,151

there are some marked variations for the same species between the different site classes. No significant correlation could, however, be established between phosphorus content and site quality.

According to Tamm (27) and Withrow (33) light intensity would enhance phosphorus absorption by plants, and, from examination of the data of Scott (25), it would seem that different sites exhibited significantly different average phosphorus values. For forest plants growing under natural conditions of site density, our results show no evidence to support this assumption.

The content of phosphorus appears to be lower in forest plants than in common agricultural plants studied by Miller (19).

6. Total Cation Content

As it has been shown by numerous workers (2, 12, 19, 21, 29) that a balance exists between the various elements in plant tissues, a better understanding of mineral nutrition of these forest plants could be gained by the study of their total cation content.

According to the results presented in Table VII, the plants analyzed can be separated into two groups: (*a*) the herbs (*Cornus canadensis* and *Oxalis montana*), and (*b*) the Ericaceae shrubs and the mosses, the former having a higher concentration of cations than the latter.

As mineral elements are mostly contained in the leaf tissues rather than in the stem (4, 11, 15, 17), the annual leaf fall should enrich the organic portion of the soil. In the case of *Cornus* and *Oxalis*, where cations are abundant, the enrichment of the humus layers should be substantial. Table IX and Fig. 1 indicate that forest stands where these two species are most abundant belong to the best site classes. The importance of shrubs and mosses for the enrichment of the humus layer depends on their abundance rather than on the content of mineral cations in their leaf tissues.

TABLE VII

COMPARISONS OF THE TOTAL CATION[a] CONTENT OF PRINCIPAL PLANT INDICATORS BETWEEN DIFFERENT SITE CLASSES (IN P.P.M.)

Species	Site I	Site II	Site III	Site IV
Herbs				
Cornus canadensis L.	17,615	14,977	16,281	16,952
Oxalis montana Raf.	15,604	15,053	—	—
Dwarf shrubs				
Chiogenes hispidula (L.) T. and G.	9,915	9,912	8,910	10,461
Kalmia angustifolia L.	—	7,409	7,226	7,000
Ledum groenlandicum Oeder	—	5,426	6,591	5,932
Vaccinium pennsylvanicum Lam.	—	6,671	7,422	6,809
Mosses				
Hypnum crista-castrensis Hedw.	9,026	9,171	8,688	9,790
Calliergonella Schreberi (Bry. Eur.) Grout	6,661	7,666	6,991	6,832
Sphagnum sp.	8,890	7,362	6,980	7,371
Hylocomium splendens (Hedw.) Bry. Eur.	5,836	6,542	5,586	6,608

[a]Phosphorus not included.

A statistical analysis of the results has revealed that the total content of mineral cations in either mosses or herbs from different site classes does not vary significantly, but that the amount of mineral elements in the leaves of these plants does vary very significantly according to the species.

Humus Layer Analysis

According to Lafond (14) the humus layer in the boreal forest is known to possess a very high exchange capacity and a low mineral cation saturation. Some of the cations released by the leaves may therefore increase the cation saturation and consequently the fertility level of the humus layer. However, this process of site improvement resulting from decomposition of plant residue and synthesis of extracellular compounds by microorganisms (3) is somewhat impeded by the acidity of the humus layer, which is maintained at a high level by the continual addition of coniferous needles having, according to Plice (22), a very low buffering capacity. Although the absolute value of exchangeable mineral cations may increase, the pH value may be lowered owing to the addition of material having a still greater exchange capacity mostly saturated with hydrogen ions.

It is suggested that the process of humus enrichment through leaf decomposition exerts its influence mostly during the pioneer and transitional stages of forest stand development. When equilibrium is reached between the soil, the climate, and the vegetation, the amount of mineral added to the soil by decay of plant tissues is balanced by the amount lost by leaching and the fertility level would remain constant.

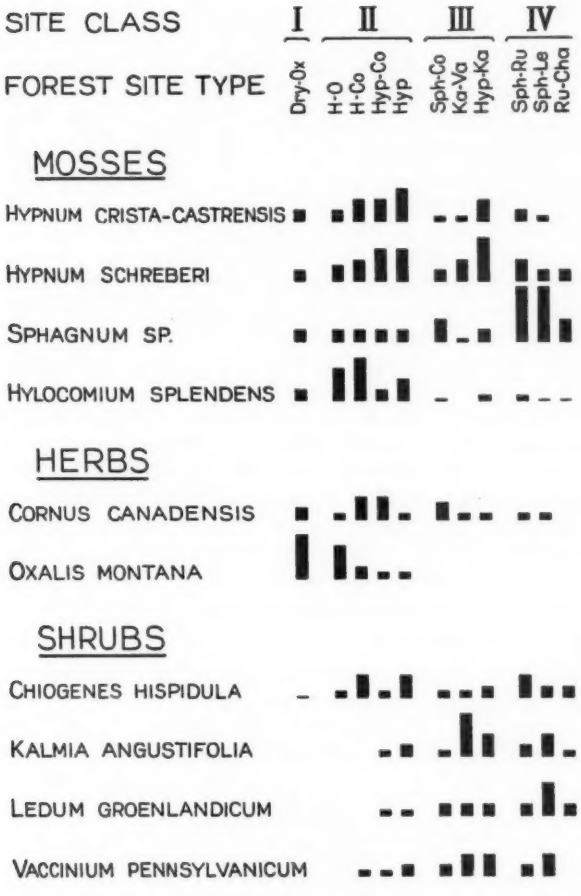

FIG. 1. Relative average abundance of the principal plant indicators in each forest type.

TABLE VIII

TOTAL AND EXCHANGEABLE QUANTITY OF NUTRIENT ELEMENTS IN THE HUMUS OF VARIOUS FOREST SITE CLASSES AND pH OF THESE HUMUS LAYERS

Site	pH		Humus elements in parts per million						
			Ca	Mg	K	Fe	Mn	P	Total
I	4.9	Total	3,670	717	1,010	1,570	316	70	7,353
		Exch.	2,600	312	468	3	[a]	63	3,446
II	4.4	Total	1,520	569	1,377	618	233	97	4,414
		Exch.	1,420	252	390	19	8	63	2,152
III	3.9	Total	1,850	1,025	551	730	[a]	53	4,209
		Exch.	960	156	273	8	[a]	30	1,427
IV	3.7	Total	1,140	711	1,384	915	18	[a]	4,168
		Exch.	900	96	117	9	[a]	[a]	1,122

[a]Amount too small to measure.

The humus layers of the stands where the leaves of the plants were collected were also analyzed for their nutrient content. Table VIII shows the comparison between the exchangeable elements and the total amount of these same nutrients contained in the organic matter, and also indicates the pH values of these layers.

From examination of these data, it is evident that the accumulation of the nutrients in the humus is closely related to the site class and also to pH values. This could be explained from the more favorable conditions for humus transformation prevailing in the best sites and stimulating the release of tissue-bound ions. It should be noted that in site class I the total amount of nutrients is almost twice as high as in the other site classes. Fig. 1 indicates that in site class I the combined abundance of *Cornus canadensis* and *Oxalis montana*, which were previously shown to contain a larger amount of cations, is dominant in the stands flora.

TABLE IX

LIST OF SITE-TYPES IN ORDER OF PRODUCTIVITY AS INDICATED BY SITE INDEX[a]

Site class	Site-type	Site index[b]
I	*Dryopteris–Oxalis*	51
II	*Hylocomium–Oxalis*	41
	Hylocomium–Cornus	39
	Hypnum–Cornus	38
	Hypnum	36
III	*Sphagnum–Cornus*	35
	Kalmia–Vaccinium	33
	Hypnum–Kalmia	31
IV	*Sphagnum–Rubus*	19
	Sphagnum–Ledum	19
	Rubus–Chamaedaphne	15

[a]After Linteau (16).
[b]Based on height of dominant and codominant fir and black spruce at 50 years.

The productivity of the stands selected, as shown in Table IX, has been established using the forest site type classification proposed by Linteau (16) and it was ascertained by the determination of the site index of the stands. Site index, here, is the height at 50 years reached by the average dominant tree in the stands and it applies both to balsam and black spruce.

Conclusions

From this short study of the nutrient content of leaves and humus collected in four different forest site classes, it appears that the species analyzed from different forest communities concentrate in their tissues mineral nutrients at a level not directly correlated with the available amount of cations in the humus layer but determined by the inherent capacity of the species. Thus plants are shown to behave in nature as they have previously been found to behave in the laboratory (1, 12, 17, 18, 23, 30).

Owing to their ecological specificity, some plants are characteristically associated with certain sites or with a group of sites. Although the nutrient content of the leaf tissues is higher in certain plants than in others, for a given species it does not vary significantly from one site or site class to another. It seems likely that the abundance of a plant on a site does to a certain extent contribute to the enrichment of the humus layer.

Hence plants belonging even to the lower strata of the vegetation have a strong influence upon the development of the forest community. They build up the soil fertility and, by the physicochemical properties of their decomposed tissues, influence the fertility level of the humus layer, which is of prime importance in the development of the forest, at any rate in the Boreal Forest Region.

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CONTROL OF NUCLEAR PROCESSES BY AUXIN IN AXILLARY BUDS OF TRADESCANTIA PALUDOSA[1]

J. M. NAYLOR[2]

Abstract

Regulatory effects of auxin on mitosis and DNA synthesis were studied in lateral bud apices of *Tradescantia paludosa*. In axillary buds inhibited by the terminal bud, mitoses were absent in a group of cells constituting the initials of tunica and corpus within the apex. The DNA (Feulgen) content of interphasic nuclei in this "zone of inhibition" was uniform and no greater than that characteristic of diploid telophase (2C), despite differences in nuclear volume. In subapical bud tissues, mitoses occurred at a low rate. Upon removal of the vegetative shoot tip, this mitotic rate was increased and the previously inhibited nuclei within the apex underwent DNA doubling to 4C, then entered prophase. These results indicate that the interphase synthesis of at least one chromosomal substance (DNA) is under auxin control. Evidence is presented that mitosis is not directly inhibited by this hormone. Naphthalene acetic acid was found to substitute for natural auxin in the inhibition of DNA synthesis.

Introduction

The experiments reported here concern the action of auxin in regulating the mitotic cycle in plant cells. Previous investigations have shown that, in cambium, the natural stimulation of cell reproduction, and therefore mitosis, depends on this hormone (20, for review see (32)). Treatments with IAA,[3] an auxin component, have been found to induce mitosis in mature plant cells which rarely divide spontaneously (3, 4, 12, 14, 29). Also, mitosis without cytokinesis has been stimulated by IAA in excised tobacco pith tissue (15). Further information on the effects of auxin on nuclear processes is limited, though evidence has been provided that IAA treatment did not induce polyploidy in subapical cells in roots or shoots (3, 4, 12, 29). More recently, however, Bradley and Crane (2) have reported the induction of polyploidy in mesocarp parenchyma cells of apricot fruits, using 2,4,5-trichlorophenoxyacetic acid, a compound which elicits at least certain growth responses qualitatively similar to those produced by IAA (31).

The present cytological experiments were undertaken in order to clarify whether auxin has a function in the natural control of interphase growth processes in nuclei of meristematic cells. Axillary buds were chosen for these experiments for the following reasons. Prior to floral induction, bud growth in some species is almost completely suppressed by auxin which is transported downward in the stem from its source in the terminal bud and young leaves.

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[3]Indole-3-acetic acid.

Decapitation of the plant removes the source of the hormone and relieves growth inhibition in the upper buds on the remaining stump, while IAA or NAA[4] applied to the cut surface of the stem substitutes for the inhibiting influence of the shoot tip (6, 11, 19, 30, 32). Where lateral bud growth is virtually absent for periods of several months, as in *Tradescantia paludosa*, obviously both growth and reproduction of cells in the bud are suppressed. Thus, considered at the cell level, bud inhibition constitutes a reversible control of cell multiplication in a meristem and therefore offers unusual opportunities for investigating the natural regulatory effects of auxin on processes in the mitotic cycle.

There is much evidence from microspectrophotometric studies showing that the DNA content of individual nuclei is doubled during the interphase reproduction of chromosomes in dividing cells (5, 16, 24, 25, 34; reviews 26, 33). Expressed in arbitrary units (24), the amount of DNA in the diploid nucleus is 2C at telophase and 4C at prophase. In general, these values are constant for a given organism. Assuming the complete absence of mitosis in the inhibited bud promeristem, the suppression of DNA synthesis by auxin therefore would be indicated if all nuclei contained less than 4C amount and if an increase to 4C, followed by mitosis, occurred on the removal of the auxin supply.

Such a relationship would indicate the participation of auxin in the natural control of chromosome reproduction and would furthermore suggest that this action in axillary buds is the basis of mitotic inhibition. Conversely, a preponderance of non-dividing nuclei with 4C or higher DNA values in the absence of mitosis would suggest that chromosome reproduction continues though mitosis is prevented in the auxin-inhibited cells.

Materials and Methods

Various species have been used in previous studies of bud inhibition, and one of these, *Vicia faba*, was used in the preliminary work in these experiments. However, it was thought that the incomplete apical dominance in this species would complicate the cytological interpretation of bud inhibition since the distinction between "inhibited" and "active" meristems must depend on clear-cut differences in mitotic rate. In *Tradescantia paludosa* this difficulty is minimized since relatively little bud elongation occurs in vegetative plants below the upper six inches of the shoot. Plants from a single clone of this species were used in all experiments reported here.

Tissues used in DNA measurements were fixed in Navaschin's fluid for 18 hours, embedded in Tissuemat, and sectioned at 18 μ. The tissue was hydrolyzed in 1 N HCl for 10 minutes at 60° C. after being brought to that temperature in distilled water. The material was then transferred to the Feulgen reagent for 1 hour at room temperature, dehydrated in an ethanol–xylol series, and mounted in Harleco synthetic resin ($N_D = 1.5150$).

[4]α-Naphthalene acetic acid.

The dye content of stained interphase nuclei was measured by the method outlined by Pollister and Ris (17) and Swift (24) using a microspectrophotometer in which the light was isolated by a monochromator designed by Dr. K. Patau. Some of the nuclei were too densely stained for accurate measurements at the maximum of the Feulgen absorption curve, hence the off-peak wave length of 615 mμ was used in all measurements. The DNA content of individual nuclei was calculated as D^2E, where D is nuclear diameter, and E is the extinction of a plug (diameter no greater than one-third D) through the nuclear axis. Variations in line current which produce slight momentary deviations in galvanometer response introduce corresponding errors in E. This effect was minimized by obtaining two values for E for each nucleus and discarding those replications which differed by more than an arbitrary amount (in this case 0.007).

In the experimental inhibition of lateral bud growth, the upper six inches or more of the shoot was removed, leaving two inches of stem above the uppermost bud. Two per cent NAA in lanolin was then applied to the cut surface. During the treatment period, a small segment was removed daily from the top of the stump and the growth regulator reapplied. This was done in order to ensure a continuity of fresh tissue for the passage of the growth substance within the stem. Twelve plants were treated in this way for 8 days; an equal number of decapitated plants were used as checks.

Observations

Feulgen stained preparations revealed a striking differentiation among the cells in the upper portion of the inhibited buds (Figs. 5, 6). The dome-shaped apex distal to the youngest leaf primordia consisted of a single outer layer of cells with rather densely stained interphase nuclei and beneath this, an ovoid group of cells in which the nuclei were relatively large and lightly stained. No mitoses were found in this distal portion of the apex, which will be called the "zone of inhibition". The lower margins of this zone were quite clearly marked since interphase nuclei in the adjoining apical tissue were relatively heterogeneous in size and densely staining, and a few mitotic divisions occurred throughout, with a greater frequency at the leaf primordia. Comparisons of Feulgen preparations with sections stained with safranin and fast green indicated that the zone of inhibition includes the initials of both tunica and corpus (Figs. 5, 6, 10).

Following the removal of the upper portion of the stem, elongation of the uppermost bud could be observed at 5 days, though cytological changes in the bud began much earlier. Two days after decapitation, mitotic activity in the leaf primordia accelerated, and at the same time, the staining intensity of the nuclei in the zone of inhibition was increased (Fig. 7), while at the lower margin of this zone, a few nuclei had entered prophase. In buds fixed 3 or more days after decapitation, the meristem had the essential characteristics of the active vegetative shoot apex (Figs. 8, 9).

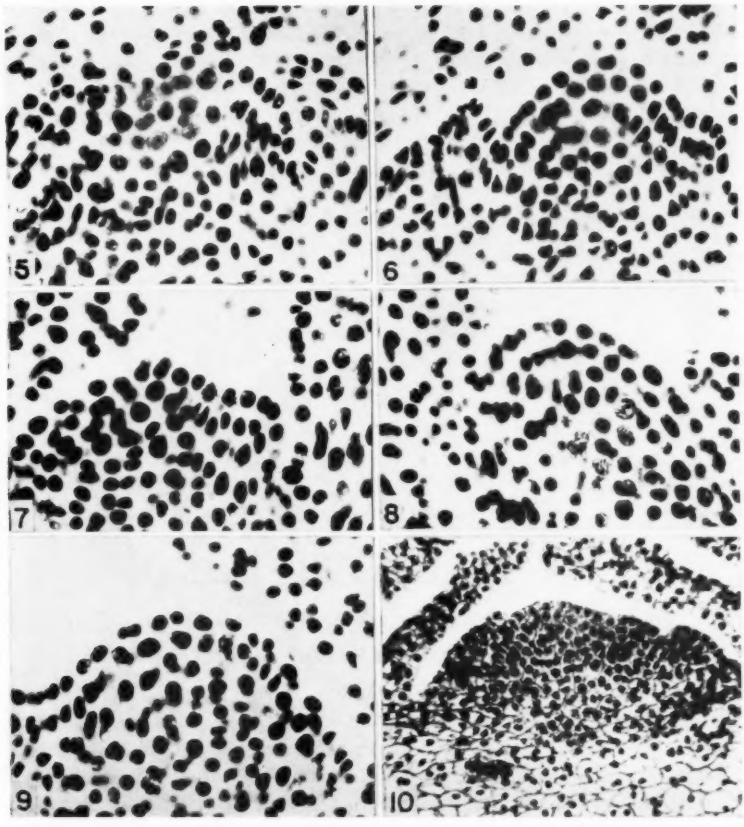
It was clear from these observations that the physiological suppression of cell reproduction was not uniform throughout the bud since a few mitotic figures were observed in the subapical portions of inhibited buds. Moreover, the relationships of size and staining intensity of "held" nuclei in the apical portion suggested that these contained very similar amounts of DNA. Furthermore, the sharp increase in nuclear staining intensity in the bud apex following the release of inhibition and preceding the onset of prophase suggested also that the "released" nuclei underwent DNA synthesis during this interval.

For DNA measurement, buds were chosen which exhibited a large, well defined zone of inhibition. In two or three serial longitudinal sections through the central portion of the apex in each bud, all intact nuclei within the margins

TABLE I

DNA CONTENT OF INDIVIDUAL NUCLEI IN INHIBITED BUDS AND STEM CORTEX

Buds		Cortex	
Bud No.	DNA content (D^2E)	Sample No.	DNA content (D^2E)
	608		634
	656		681
1	624		720
	592		694
	576		641
	649	1	520
			661
	612		599
2	663		541
	539		573
			607
	778		
3	633		674
	766		631
	568		695
		2	754
	694		673
	572		716
4	616		648
	615		627
	550		
	692		
	601		
5	726		
	632		
	714		
	574		
$M=635$; S.E. $=13.5$		$M=647$; S.E. $=13.9$	
Variation between buds: F (d.f. 4,19) $=1.31$ F ($P=.05$) $=2.90$		Variation between cortex samples: F (d.f. 1,17) $=3.92$ F ($P=.05$) $=4.45$	
Variation between buds and cortex: F (1,41) $=0.34$ F ($P=.05$) $=4.07$			



FIGS. 5–10. Longitudinal sections through axillary buds and shoot meristem.

5. Naturally inhibited bud, showing zone of inhibition. Feulgen.
6. Bud from decapitated plant treated with naphthalene acetic acid for 8 days. Note the zone of inhibition. Feulgen.
7. Released bud, fixed 2 days following removal of the shoot tip. Feulgen.
8. Released bud, fixed 3 days following removal of the shoot tip. Feulgen.
9. Vegetative shoot meristem. Feulgen.
10. Naturally inhibited bud, showing two-layered tunica. Safranin and fast green.

of the zone of inhibition were measured, excepting those with extraneous fragments of other (cut) nuclei lying in the light path.

DNA measurements were made in five naturally inhibited apices, and in the stem cortex adjoining two of these. DNA values for individual nuclei, together with a summary of the statistical analysis are given in Table I. Differences among buds and among cortex samples, and between the two tissues (buds and cortex) were below significance at $P = .05$. In both buds and cortex, the coefficient of variation* was close to 10% (10.4% and 10.7% respectively). Since errors in measurement can be expected to account for at least one-third of this variation (28), actual differences among nuclei were probably small.

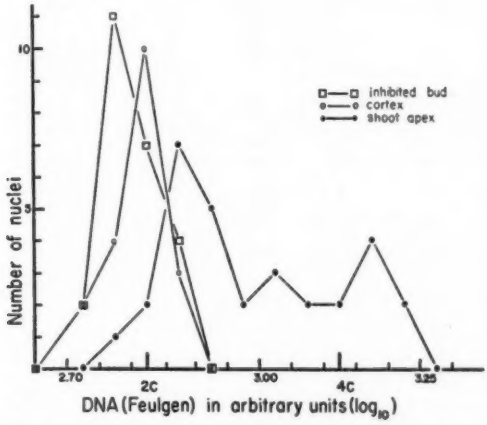


FIG. 1. Distribution of DNA measurements in inhibited bud apices, stem cortex, and terminal bud meristem.

Comparable frequency distributions of DNA values of interphase nuclei in inhibited buds, cortex, and vegetative shoot apex are shown in Fig. 1, indicating that the distributions for buds and cortex fall at the lower limit of the curve representing the shoot apex. Therefore, it is evident that the DNA content of these nuclei did not exceed that of diploid telophase (2C). While the above data do not indicate that any cortical nuclei contain higher amounts of DNA than 2C, large densely staining nuclei with 4C or intermediate values were occasionally found by searching. These were not plotted in Fig. 1, since they do not constitute a random sample.

Within 3 days after the removal of the shoot tip, an upward shift in DNA content occurred in the apical nuclei of released buds. Combined data from two such buds and the adjacent stem cortex fixed at this stage (Fig. 2) show DNA values in the bud apex ranging from 2C to 4C. Though the majority of these previously inhibited nuclei had reached 4C at this stage, prophase figures were rare, indicating that DNA was doubled simultaneously in a large

*Standard deviation in percentage of the mean.

proportion of the nuclei or that prophase did not begin immediately on the completion of DNA synthesis. The fact that these nuclei ultimately divide was shown in buds fixed after longer periods of release.

In decapitated plants supplied with NAA, no elongation of axillary buds was observed during the period of treatment, while considerable axial growth occurred in the severed internode. In contrast, elongation of the upper buds

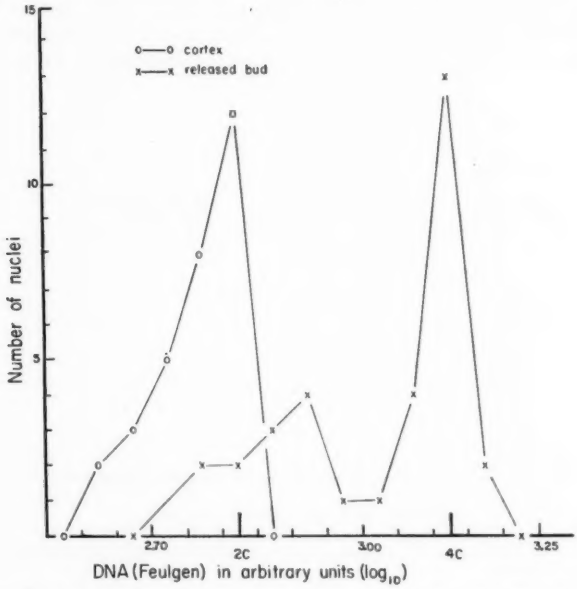


FIG. 2. Distribution of DNA measurements in apices of released axillary buds, 2 days after removal of the shoot tip.

was evident in control plants within 5 days after removal of the shoot tip, though there was little if any stem elongation. No evidence of mitosis was found in the apical portions of any of the six treated buds examined, and with one exception the zone of inhibition in each was clearly distinguishable.

DNA measurements were carried out in four treated buds and the stem cortex adjoining two of these (Table II). Complete inhibition of DNA synthesis was maintained throughout the experiment in buds 3 and 4, as all values are grouped at the 2C level, though significantly higher amounts were recorded in buds 1 and 2 (Fig. 3, Table II). This difference between buds may have resulted from an unknown error in the assay procedure or from a limited synthesis of DNA in buds 1 and 2. However, even if the latter possibility is correct, it is clear that no general release of inhibition occurred, since DNA values approaching 4C were not found, and mitoses were absent in all treated bud apices.

TABLE II

DNA CONTENT OF NUCLEI IN BUDS INHIBITED BY NAA

Bud No.	DNA content (D^2E)	
	803	
	821	
1	769	$M = 795$
	796	
	704	
	709	
	874	
	785	
2	834	
	855	
	617	
	606	
	743	
3	705	$M = 671$
	687	
	564	
	729	
	705	
4	647	
	704	

t (buds 1,2:3,4) = 4.80 t (d.f. 18, $P = .05$) = 2.10

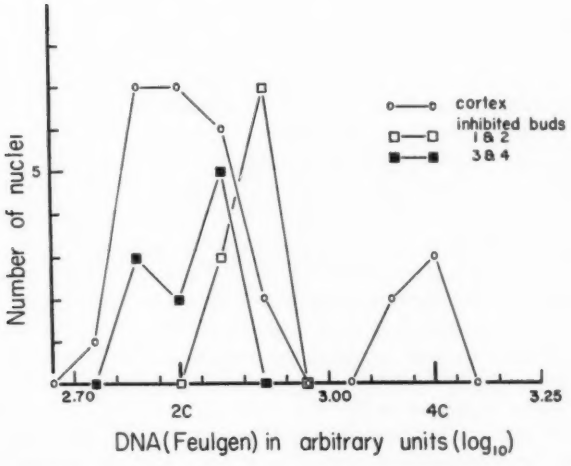


FIG. 3. Distribution of DNA measurements in axillary buds and stem cortex of decapitated plants treated for 8 days with α-naphthalene acetic acid.

Whereas NAA treatment effectively substituted for natural auxin of the shoot tip in inhibiting DNA synthesis in axillary bud apices, the behavior of the stem cortex in normal and treated plants was quite different. In the latter, 4C nuclei were relatively much more frequent, indicating a greater incidence of DNA doubling (Figs. 1, 3). This is correlated with the acceleration in internode elongation observed in these treated plants.

Discussion

It is well established that lateral bud inhibition is auxin controlled (32), though the mechanism is still in some doubt. Kefford (13) has recently shown that a growth inhibitor (β) which is present in all parts of the shoot occurs in relatively higher concentration in the axillary buds in *Vicia faba*. Hemberg has also demonstrated a growth inhibitor in dormant buds of *Fraxinus* (10), and in "resting" potato tubers (9). These substances are not identical with auxin. Moreover, earlier work by Snow (21, 22) gave evidence that lateral bud growth is inhibited by a substance formed in response to auxin in the shoot. It is possible, therefore, that the cytological observations presented here do not refer to a direct effect of auxin and, accordingly, that growth processes affected by auxin in the axillary bud are not identical with those in other meristematic tissues. However, whether the effect is direct or indirect, it is clear that in this material, auxin blocks the mitotic cycle by suppressing the interphase synthesis of at least one chromosomal substance (DNA). This is shown by the uniformity in DNA content (2C) of inhibited nuclei (Table I, Fig. 1), and by the fact that the DNA content of these cells is doubled prior to the onset of prophase when inhibition is relieved (Fig. 2). The DNA content of inhibited nuclei fall at the lower extreme of the 2C–4C range found in the diploid, dividing shoot meristems. However, the lower frequency maximum of the curve representing shoot meristem does not coincide with those for buds and cortex (Fig. 1). This discrepancy probably resulted from an error in preparing the shoot material for measurement, or from a consistent error in the measurements in this tissue. The existence of a real difference between tissues, constituting a departure from DNA constancy is possible, though unlikely, in view of the considerable evidence to the contrary (26, 33). Nuclei in the apices of inhibited buds are therefore considered as 2C.

The absence of both 4C values and mitotic figures in the inhibited promeristems examined in this study indicates that if DNA doubling occurs occasionally, it is regularly followed by mitosis, thus restoring the relatively stable 2C condition. For the same reason, during the onset of inhibition in the young axillary promeristem, those nuclei in the process of DNA doubling or mitosis, must complete those processes, though 2C nuclei rarely initiate synthesis. Evidently, DNA synthesis is blocked in these nuclei, though mitosis is not directly inhibited. Therefore the evidence (4, 12, 29) that IAA influences mitosis but not chromosomal reproduction in "mature" subapical cells of roots and stems cannot be taken to indicate a general role of auxin in meristematic cells.

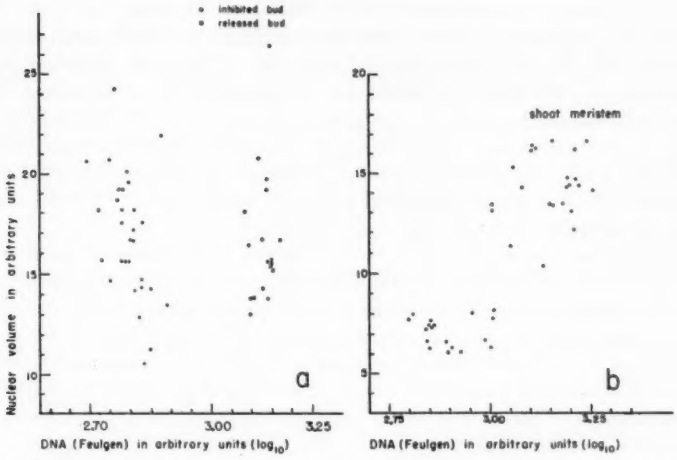


FIG. 4. Relationship between DNA content and volume of interphasic nuclei in the apices inhibited and released buds (4*a*) and in the dividing shoot meristem (4*b*).

These results do not show that the inhibition system specifically regulates DNA synthesis. It is quite possible that this effect is merely one manifestation of a general stabilization of synthetic processes in the nucleus or the cell as a whole. Interestingly, however, while DNA is held at the 2C level, inhibited cells, except those in the outer layer of the tunica, contain large nuclei which, on release of inhibition, undergo DNA doubling and enter prophase with no increase in volume (compare Figs. 4*a*, 4*b*). This may reflect a specificity in the effects of auxin. Thus, the normal interphase doubling of those chromosomal substances other than DNA which contribute appreciably to nuclear volume may proceed though the amount of DNA is held constant during inhibition.

Using the Millon reaction, Swift (27) has found a direct proportionality between protein content and volume of interphase nuclei in *Allium* root tips. A similar relationship has been reported in animal tissues by Schrader and Leuchtenberger (18) and Alfert and Bern (1). Thus, proportionate increases in volume and protein occur during interphase in dividing cells. Accordingly, the large inhibited nuclei which have the typical prophase volume would be expected to contain the amount of protein found in prophase following the release of inhibition. This has not been extensively tested, though Woodard (unpublished) has found some evidence (Millon reaction) that the synthesis of protein is also suppressed in these inhibited nuclei. If this is true, the large volume of the inhibited nuclei is largely determined by proteins such as histone, which does not strongly influence the Millon reaction, or by factors other than protein content.

The zonation in the inhibited bud apex is interesting for several reasons. It is apparent that inhibition is not uniformly expressed throughout the bud,

since a few mitotic figures were usually found in the subapical tissue below the zone of inhibition. Thus, inhibition of DNA synthesis and mitosis is complete only at the morphological bud tip. This fact supports Snow's contention (21, 22) that bud inhibition is dependent on a substance that is formed in response to auxin in the stem and which moves acropetally with respect to the stem axis. This substance would be expected to accumulate at the bud apex. Accordingly, growth inhibition would be relatively greater in this portion of the bud, assuming a direct proportionality between concentration of the substance and degree of inhibition.

Stant (23) has described a pattern of zonation in the shoot apices of several monocot species resembling the condition observed in the lateral buds in this study. In these species, a terminally located group of apical cells constitutes the initials of both tunica and corpus. These cells, like those in the zone of inhibition of *Tradescantia* lateral buds, contain unusually large, lightly stained nuclei and divide less frequently than adjoining cells in the apex. In certain gymnosperms too, the corpus initials are conspicuously large and divide at a lower rate than the other subterminal cells (7, 8).

Whether this zonation in the terminal bud is regulated by the local accumulation of growth-regulating substances is unknown (35), though the present study indicates that the similar condition in the axillary buds is controlled directly or indirectly by auxin. If, as Snow concludes, a substance formed in the presence of auxin in the stem moves upward into the axillary bud and suppresses growth, it may also reach the terminal bud and elicit a qualitatively similar response. The fact that the mitotic rate is lowest at the extreme distal portion of the apex in both *Tradescantia* axillary buds and in the terminal buds observed by Stant may therefore reflect the effects of the same inhibitor.

The experiments with naphthalene acetic acid indicate that this growth regulator substitutes for the influence of the shoot tip in maintaining the inhibition of DNA synthesis and mitosis in the apical cells of lateral buds (Fig. 6). This provides additional evidence that these processes are regulated by auxin, since in general, growth responses to naphthalene acetic acid and natural auxin are qualitatively very similar. However, the responses of stem cortex and axillary buds were quite different, as the inhibition of DNA synthesis by NAA was not found in the former tissue adjacent to experimentally inhibited axillary buds. In fact, the relative incidence of 4C nuclei in the cortex of treated and normal plants (Figs. 1, 3) suggests that the NAA treatment *stimulated* DNA synthesis in this tissue.

Thimann has observed that within a certain range, concentrations of auxin sufficient to inhibit lateral bud elongation may stimulate stem growth (32). The present results suggest that a similar relationship holds for DNA synthesis.

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SOME EFFECTS OF INDOLEACETIC ACID AND MALEIC HYDRAZIDE ON THE RESPIRATION AND FLOWERING OF WHEAT[1]

M. SHAW, D. J. SAMBORSKI,[2] AND A. OAKS[3]

Abstract

Wheat seedlings were grown in sand culture in 1 qt. crocks. Maleic hydrazide (10 mg. per crock per day) prevented flowering and increased the oxygen consumption per unit dry weight of the first leaves by 20 to 50%, but did not alter the C_6/C_1 ratio, which was measured by incubating leaf disks with glucose-6-C^{14} and glucose-1-C^{14}. Indoleacetic acid (0.5 mg. per crock per day) increased oxygen uptake by only 10 to 15%, but stimulated flowering and apparently lowered the C_6/C_1 ratio. The C_6/C_1 ratio of leaf disks was approximately halved by 2 hours pretreatment with 5.0 and 8.3 p.p.m. of indoleacetic acid, mainly because of a decrease in the recovery of C_6.

The results are discussed briefly in relation to the effect of obligate parasites in increasing the indoleacetic acid content and in lowering the C_6/C_1 ratio of susceptible cereal leaves.

Introduction

The C_6/C_1 ratio for wheat and barley leaves is lowered by infection with *Puccinia graminis* and *Erysiphe graminis* (8). Infection also apparently raises the free indoleacetic acid content of the leaves of susceptible varieties (9). Maleic hydrazide breaks the resistance of Khapli wheat to stem rust (6). This paper describes some effects of maleic hydrazide and indoleacetic acid on oxygen uptake and the pathway of glucose dissimilation in the first seedling leaf of wheat and on flowering.

Methods

Little Club and Khapli wheats were grown in sand culture in 1 qt. crocks as described in (6). When the first leaves were fully emerged, the plants were thinned to five per crock and the daily addition to each crock of either 10 mg. of maleic hydrazide (Eastman Organic Chemicals, practical grade) or 0.5 mg. of indoleacetic acid (Nutritional Biochemicals Corp.) was started and continued for at least a month. It is important to note that the crocks were thoroughly flushed each day with tap water.

Respiratory measurements on leaf pieces were made with a Warburg apparatus at $25 \pm 0.02°$ C. (5, 8). The measurement of C_6/C_1 ratios was carried out as described in (8).

Results

Flowering

The general effects of maleic hydrazide and indoleacetic acid on the plants have already been described (6, 7). Maleic hydrazide prevented flowering (6); on the other hand, indoleacetic acid stimulated it as illustrated by Fig. 1

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and the data in Table I. These observations are in agreement with the finding that α-naphthaleneacetic acid stimulates flowering in Wintex barley and Petkus rye (4).

TABLE I

THE EFFECT OF INDOLEACETIC ACID ON FLOWERING IN KHAPLI WHEAT

Days of treatment	Length of inflorescence, mm.	
	IAA (0.5 mg./crock/day)	Control
30	25.2 ± 11.35	14.0 ± 4.32
40	63.3 ± 5.34	49.4 ± 5.16

Respiration

It has been shown that maleic hydrazide causes marked metabolic disturbances in the wheat leaf (6, 7). The data in Table II show that oxygen uptake was increased but that the C_6/C_1 ratio was not altered by maleic hydrazide. Since dry weight per unit area is more than doubled after 12 days of treatment with maleic hydrazide (see 6), the increases in oxygen uptake per unit area were at least double those shown in Table II. Indoleacetic has little effect on dry weight (6) and was less effective than maleic hydrazide in raising oxygen consumption. On the other hand, after 12 days of treatment with indoleacetic acid, the C_6/C_1 ratios were distinctly lowered (Table II).

In view of these results disks cut from the first leaves of Little Club were incubated with a series of concentrations of indoleacetic acid (1.7 to 16.6 mg./liter in 0.05 *M* KH_2PO_4) for 2 hours, after which glucose-1-C^{14} and glucose-6-C^{14} were added and oxygen uptake and the C_6/C_1 ratios measured over a second 2-hour period. The results are given in Table III. A respiratory peak (about 15% stimulation) was observed at an indoleacetic acid concentration of 3.3 mg./liter and, up to this level, there was no definite

TABLE II

THE EFFECT OF INDOLEACETIC ACID AND MALEIC HYDRAZIDE ON THE OXYGEN CONSUMPTION AND C_6/C_1 RATIO FOR THE FIRST LEAVES OF LITTLE CLUB AND KHAPLI

Species	Age, days	Days of treatment	Control		IAA*		MH†	
			O_2 uptake	C_6/C_1	O_2 uptake	C_6/C_1	O_2 uptake	C_6/C_1
Little Club	15	5	23.1	0.45	23.7 (3)	0.56	26.3 (14)	0.47
	22	12	19.6	0.43	22.5 (15)	0.38	23.5 (20)	0.46
Difference				0.02		0.18		0.01
Khapli	13	5	28.5	0.61	26.6 (−7)	0.59	30.0 (5)	0.62
	20	12	17.5	0.56	19.5 (11)	0.37	26.3 (50)	0.56
Difference				0.05		0.22		0.06

NOTE: O_2 uptake = μλ/10 mg./hr. Figures in parentheses give stimulation as per cent of controls.
*0.5 mg. per crock per day.
†10 mg. per crock per day.

PLATE I

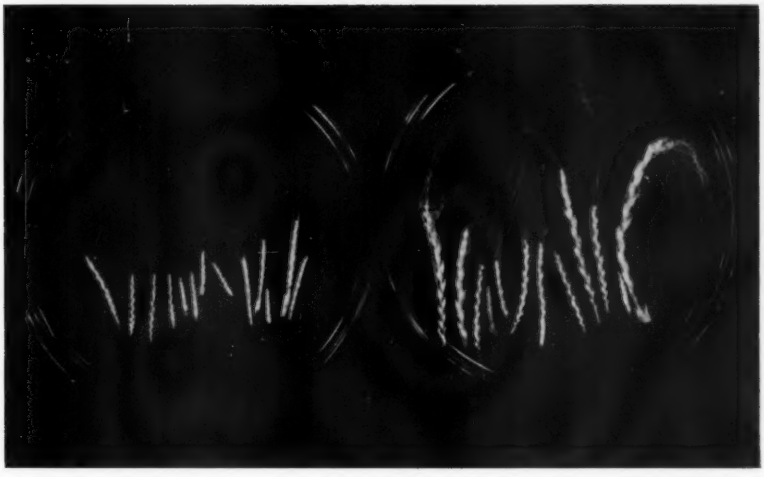

FIG. 1. The effect of indoleacetic acid on the length of the inflorescence of Khapli wheat. Left, untreated controls. Right, treated with 0.5 mg. indoleacetic acid per crock daily for 30 days after emergence of the first seedling leaf.

TABLE III

THE EFFECT OF INDOLEACETIC ACID ON THE OXYGEN UPTAKE AND C_6/C_1 RATIO OF DISKS CUT FROM THE FIRST LEAVES OF LITTLE CLUB

IAA		Relative radioactivity from glucose in CO_2 (c.p.m./10 mg. dry wt./2 hr.)			O_2 uptake	
mg./liter	μg./flask*	C_6	C_1	C_6/C_1	per 10 mg. dry wt./hr.	%
16.6	50	37	205	0.18	22.7	86.3
8.3	25	85	425	0.20	26.4	100.4
5.0	15	131	422	0.31	26.1	99.2
3.3	10	219	465	0.47	30.2	114.8
1.7	5	217	540	0.40	27.9	106.1
0	0	213	403	0.53	26.3	100.0

*100 leaf disks (diam. 2.8 mm.) in 3 ml. 0.05 *M* KH_2PO_4 per flask. Two flasks with glucose-1-C^{14} and two with glucose-6-C^{14} were used for each determination of the C_6/C_1 ratio and oxygen uptake.

change in the C_6/C_1 ratio. At 5.0 and 8.3 mg./liter the respiratory stimulation was abolished and the C_6/C_1 ratio was drastically lowered. While the recovery of C_1 was slightly increased by indoleacetic acid, the lowering of the C_6/C_1 ratio was due mainly to the decreased recovery of C_6. The data also suggest that the lower concentrations of indoleacetic acid may have slightly increased the consumption of glucose.

Discussion

Gibbs and Beevers (2) reported that the C_6/C_1 ratio for root, stem, and leaf tissues of a number of species fell as the tissue aged. They assumed that the C_6/C_1 ratio gave directly the 'maximum' fraction of the respiration which is carried through the Embden – Meyerhof – Parnas – tricarboxylic acid pathway. They therefore concluded that the pentose phosphate pathway plays an increasingly important role in respiration as tissues age and differentiate. The assumptions and limitations of the 'C_6/C_1 technique' have been discussed by Axelrod and Beevers (1).

Whatever the final interpretation of the C_6/C_1 ratio may be, the results in Table II support the experimental findings of Gibbs and Beevers (2). When growth and development are stimulated by treatment with indoleacetic acid, as evidenced by the stimulation of flowering (Table I), the C_6/C_1 ratio for the first leaves falls more rapidly than in untreated plants or in plants whose growth and development have been inhibited by maleic hydrazide. Whether there is any relationship between floral initiation and production and respiratory pathways in the leaves is an interesting question on which information is lacking.

Gibbs and Beevers (2) found that 2.4 p.p.m. of indoleacetic acid stimulated the growth of corn coleoptiles by about 25% and oxygen uptake by about 26%, but had no appreciable effect on the C_6/C_1 ratio. While the data in

Table III confirm the finding that low concentrations (1.7 and 3.3 mg./liter) of indoleacetic acid do not affect the ratio, they show that somewhat higher concentrations do. The concentration of free extractable indoleacetic acid in the first leaves of wheat appears to be of the order of 1 μg. per kilogram fresh weight (9), far below the exogenous concentrations which affect the C_6/C_1 ratio. Exogenously supplied indoleacetic acid is oxidized by wheat leaf disks, and data obtained by one of us (A.O.) show that the total endogenous concentration of indoleacetic acid does not exceed half the exogenous concentration under our experimental conditions.

Since the experiments reported here were carried out, Humphreys and Duggar (3) have reported that treatment of corn and pea root tips with a relatively high concentration (10^{-3} M = 164 mg./liter) of indoleacetic acid for 12 hours markedly lowered the C_6/C_1 ratio. In their experiments the recovery of C_1 was almost unchanged by the treatment, the decrease in the ratio being due to the decreased recovery of C_6. They also found that 2,4-dichlorophenoxyacetic acid (10^{-3} M) strikingly lowered the C_6/C_1 ratio by increasing the recovery of C_1.

The data presented here, together with those of Humphreys and Duggar (3), suggest the possibility that indoleacetic acid may regulate the pathway of glucose dissimilation. Of extreme interest is the possibility (10) that the effects of rust and mildew in lowering the C_6/C_1 ratio reflect a hormonal control of respiratory metabolism. At present at least two observations apparently militate against any hypothesis involving a simple relationship between indoleacetic acid and the C_6/C_1 ratio in infected tissue. These are:

(*a*) the observation that in infected susceptible tissue, as in tissue treated with 2,4-D (3), the C_6/C_1 ratio falls because the recovery of the C_1 is increased (8);

(*b*) the observation that in infected, resistant tissue (Khapli wheat) the ratio falls for the same reason but the indoleacetic acid content of the leaves is apparently decreased (9).

Acknowledgments

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STUDIES ON THE EPIDEMIOLOGY OF CROWN BUD ROT OF ALFALFA IN SOUTHERN ALBERTA[1]

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Abstract

Crown bud rot is widespread in irrigated alfalfa stands in southern Alberta. *Rhizoctonia solani* Kühn, *Fusarium roseum* Link sensu Snyder & Hansen, and *Ascochyta imperfecta* Peck acting alone or in combination produce brown to black lesions on the crown buds of plants in their second and subsequent years of growth. The host plant is most susceptible to the disease in early spring after winter dormancy. Soil temperatures above 16° C. appear to reduce disease development. Relative occurrence of species isolated from infected buds indicate a succession of fungi. *F. roseum* is the predominant isolate until the third year of alfalfa growth when its numbers are approximately equalled by *R. solani*. The latter then decreases in prevalence possibly because of the antibiotic action of saprophytic species of *Trichoderma* and *Penicillium*. *F. roseum* maintains about the same degree of activity throughout the growing season whereas *R. solani* generally appears most frequently in summer-sampled alfalfa and *A. imperfecta* in spring samples.

Introduction

The crown and root diseases of alfalfa have received much study in both the United States and Canada in recent years and their causal organisms have proved to be many and varied. Broadfoot and Cormack (1) in 1941 reported a low temperature Basidiomycete, as yet unidentified, responsible for the early spring killing of grasses and legumes in Alberta. Additional evidence of the role of this pathogen and its distribution was provided in 1947 and 1948 by Cormack (4, 5). Alfalfa crowns are damaged by *Fusarium avenaceum* (Fr.) Sacc. in Alberta (2), in California (8), and by undesignated species of *Fusarium* in North Carolina (12) and in Missouri (19). Erwin (9) found that *Rhizoctonia solani* and *Stagonospora meliloti* (Lasch.) Petr. caused stem and crown bud cankers, and crown and root rot, respectively, on California alfalfa. A species of *Stagonospora* was reported by Jones and Weimer (16) as the cause of a rotting of the upper tap root and crown branches of alfalfa. Another fungus, *Diplodia* sp., was reported by Sleeth (20) in 1951 as the causal organism of crown rot in Arizona alfalfa stands.

Hawn and Cormack (14) first reported the presence of a crown bud rot in irrigated alfalfa in southern Alberta in 1952. The disease caused progressive deterioration of the crop during the second and subsequent years of growth and appeared to develop most rapidly during the early part of the growing season (13). The present paper provides information gathered to date on the epidemiology of this disease.

Description of the Disease

The crown buds of alfalfa plants grown under irrigation in southern Alberta become infected while they are at an early stage of development and in contact

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Contribution No. 1634 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
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with the soil and plant debris. Infected buds show dark brown to black lesions which enlarge and eventually cause the death and subsequent rotting which is characteristic of the disease (Figs. 1, 2, 3). The rot extends into the crowns of the plants preventing further production of vegetative buds by the invaded tissues. This leads to a reduction in the amount of forage produced. A secondary effect of this weakening of the plants is the establishment of weed growth. The dandelion (*Taraxacum officinale*) is one of the more conspicuous indicators of advanced crown bud rot. Such noxious weeds further shorten the useful life span of alfalfa under irrigation.

Crown bud rot does not spread from visible focal points as is the case in winter crown rot (5). Instead there is usually a fairly even distribution and development of the disease throughout an entire planting of alfalfa.

Method of Assessing Plant Damage

Crown bud rot damage was measured by means of a graduated scale of ratings extending from 0 for a disease-free crown to 10 for a crown devoid of living buds and shoots. For example, an alfalfa crown having two diseased buds out of a total of five would be rated $\frac{2}{5} \times 10 = 4$. If three of a total of six were diseased the rating would be $\frac{3}{6} \times 10 = 5$. By tabulating the numbers of plants in each of the categories the average amount of crown bud rot per sample could easily be calculated. Furthermore, since the rating system was based on 10, the ratings could be converted to percentage of crown buds rotted by multiplying by 10.

Methods of Field Study

Irrigated, 1- and 2-year-old alfalfa fields, 10 acres or more in area, were chosen for study. Five sample plots, each 25 ft. square, were laid out in each field in such a way as to ensure a good cross section of the field's topography. These plots were marked by stakes driven into the ground sufficiently far to prevent interference with haying implements. Finally the plots in each field were mapped carefully for ease of relocation.

Five random samples of 20 alfalfa plants each were taken from each field. They were bagged, labelled, brought into the laboratory, and rated for crown bud rot as previously described. A continuous record was kept throughout the economic life of each stand.

Three surveys were made each year. The first was made early in the spring, before any appreciable growth was evident, to provide a basis for determining the total development of crown bud rot for that year. A second set of samples was taken as soon as possible after removal of the first cut of hay (usually in early or mid-July), and the final survey was made late in the fall. The fall results, when compared with spring readings, showed the amount of disease development during the entire growing season. In addition, by comparing the fall data with those obtained the following spring, it was possible to learn whether or not this disease was one which affected plants during their dormant state (1, 5), during the growing season alone, or during both periods.

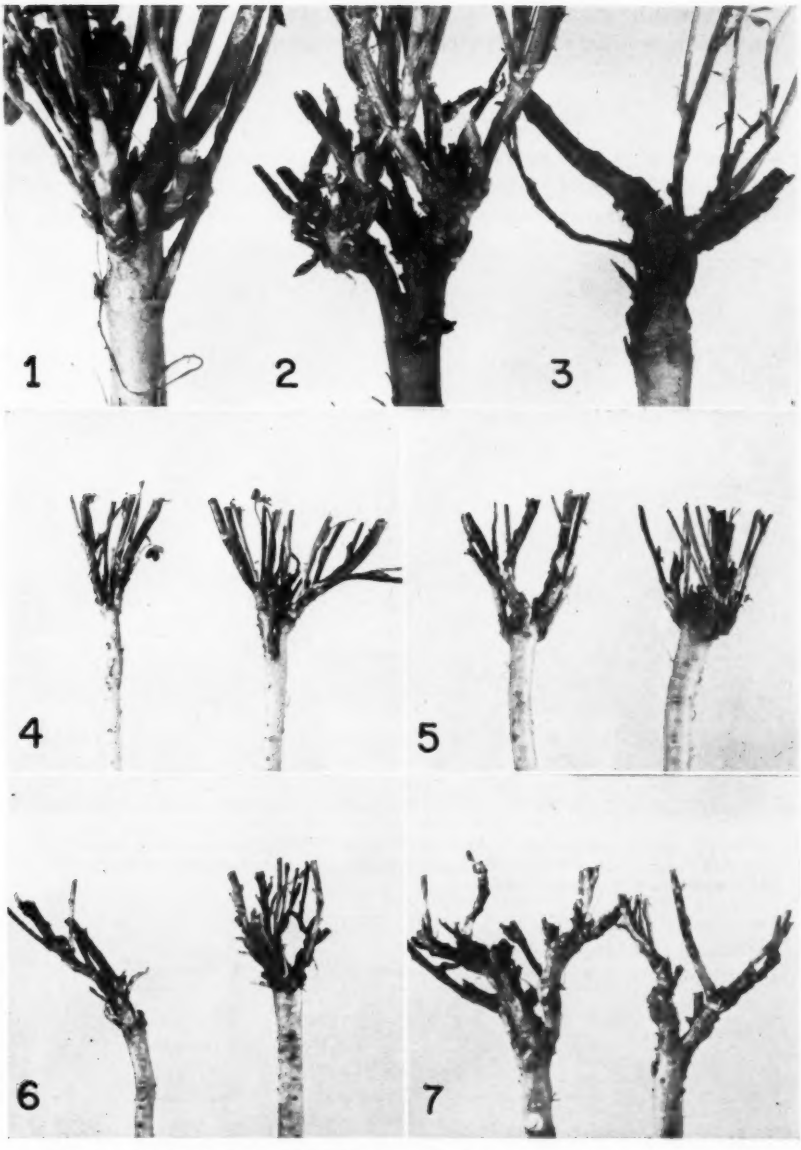

FIG. 1. An alfalfa plant with healthy crown buds.
FIG. 2. An alfalfa plant with moderate crown bud rot.
FIG. 3. An alfalfa plant severely damaged by crown bud rot.
FIGS. 4–7. Crown bud rot development in alfalfa plants 1, 2, 3, and 4 years old respectively.

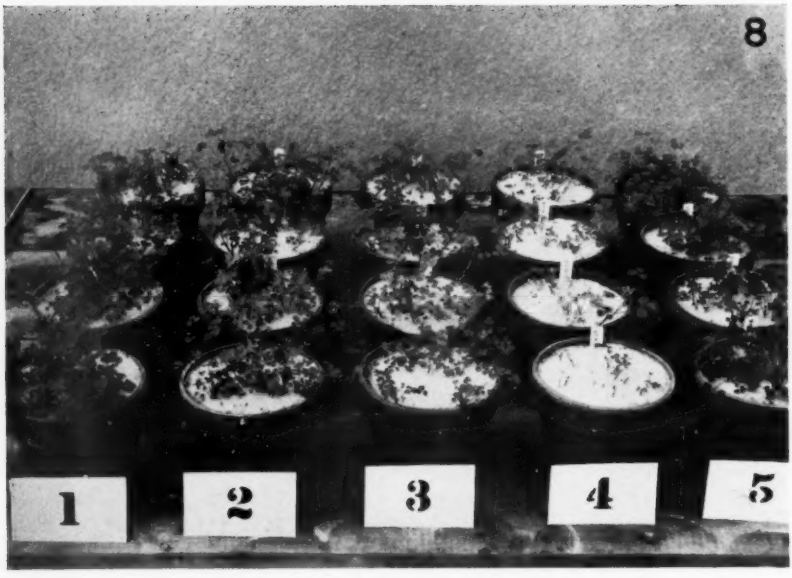

FIG. 8. Grimm alfalfa 15 days after the following treatments: (1) control, (2) inoculation with *F. acuminatum*, (3) inoculation with *F. avenaceum*, (4) inoculation with *R. solani*, (5) inoculation with *A. imperfecta*.

This method of study provided satisfactory data on seasonal and annual development of crown bud rot in fields of alfalfa. In addition, diseased specimens were taken from the samples at each survey for the purpose of isolating the causal organisms.

Disease Development in the Field

Both detailed and general types of survey have shown crown bud rot to be widespread in irrigated alfalfa in southern Alberta. However, here, as in the case of winter crown rot caused by the low temperature basidiomycete (5), a certain minimum amount of growth seems necessary before symptoms of the disease appear. Crown bud rot does not ordinarily appear until the plant is in the second year of growth.

Data collected over a 6-year period showed a positive correlation between age of stand and the incidence of crown bud rot. It was found that 20.7, 79.7, 98.5, and 100% of the plants in 1-, 2-, 3-, and 4-year-old stands, respectively, showed symptoms of the disease.

These data also showed that there was an association between the age of infected plants and the severity of the disease. This is illustrated in Figs. 4, 5, 6, 7. Disease development was slight during the first year of plant growth and again in the fourth year, with the major build-up of crown bud rot occurring in the second and third years. Average disease ratings had reached 4.3 by the fall of the fourth year when all of the plants examined were found to be diseased. In short, 43% of the average 4-year-old stands was destroyed by crown bud rot.

A comparison of seasonal crown bud rot ratings taken over a 4-year period showed that the major part of each year's disease development invariably occurred during the time between the spring and summer detailed surveys. The results are summarized in Table I.

TABLE I

AVERAGE CROWN BUD ROT DAMAGE IN ALFALFA FIELDS AT DIFFERENT SEASONS OF THE YEAR

Survey season	Age of stand in years		
	2	3	4
	Average crown bud rot rating		
Spring	0.1	2.5	3.4
Summer	1.9	3.5	4.3
Fall	2.0	3.3	4.1

To determine more exactly when this accelerated disease development occurred a series of irrigated alfalfa plots were randomly sampled during the first week of each month from April through September. Plants were assessed

TABLE II

MONTHLY DEVELOPMENT OF CROWN BUD ROT IN IRRIGATED TEST PLOTS OF LADAK ALFALFA

Month	Average crown bud rot rating		
	1952*	1953†	1954†
April	0.2	0.1	2.9
May	2.8	1.3	4.1
June	3.3	3.2	4.5
July	3.6	2.9	5.2
August	3.4	3.0	4.8
September	3.4	2.6	4.5

*Seeded in 1951.
†Seeded in 1952.

for crown bud rot and the disease development during each month was calculated. This procedure was followed for 3 years. The results are summarized in Table II.

The increase in average disease rating for May 1952 made up 81.2% of the total disease development for that year when based on the September rating. In 1953 the alfalfa plants came out of dormancy somewhat later and 48.0% of the total increase came in May and 52.0% in June. In 1954 the disease development of 75.0% during May was again more pronounced than in any other month.

Effect of Soil Temperature on Disease Development

The rapid build-up of crown bud rot during a relatively short span of time in the early part of the growing season (Table II) and seasonal differences in the rate of disease development in the field (Table I) suggested strongly that soil temperature might be one of the principal factors concerned. The work of Dickson (6) and Jones *et al.* (15) present classic evidence of the marked influence soil temperatures can exert on the development of plant diseases.

From April 1 to October 31, 1953 and 1954, a continuous record was kept of soil temperatures. Leads from duplicate thermocouples, buried at a depth of 1 in. in plots of alfalfa, were attached to a Brown potentiometer in the laboratory. Mean temperatures were calculated from this record and related to crown bud rot development in comparable plots of alfalfa for each of the months during the growing season. The results of this study are illustrated in Fig. 9.

The mean temperature 1 in. below the soil surface rose from 4.2° C. in April 1953 to the high for the year of 20.7° C. in July. In that year crown bud rot development was confined entirely to May and June (Table II and Fig. 9) when the mean temperatures were 12.4° and 15.7° C., respectively. In 1954 the soil temperatures were only 1.8° in April, 9.1° in May, and 16.2° C. in

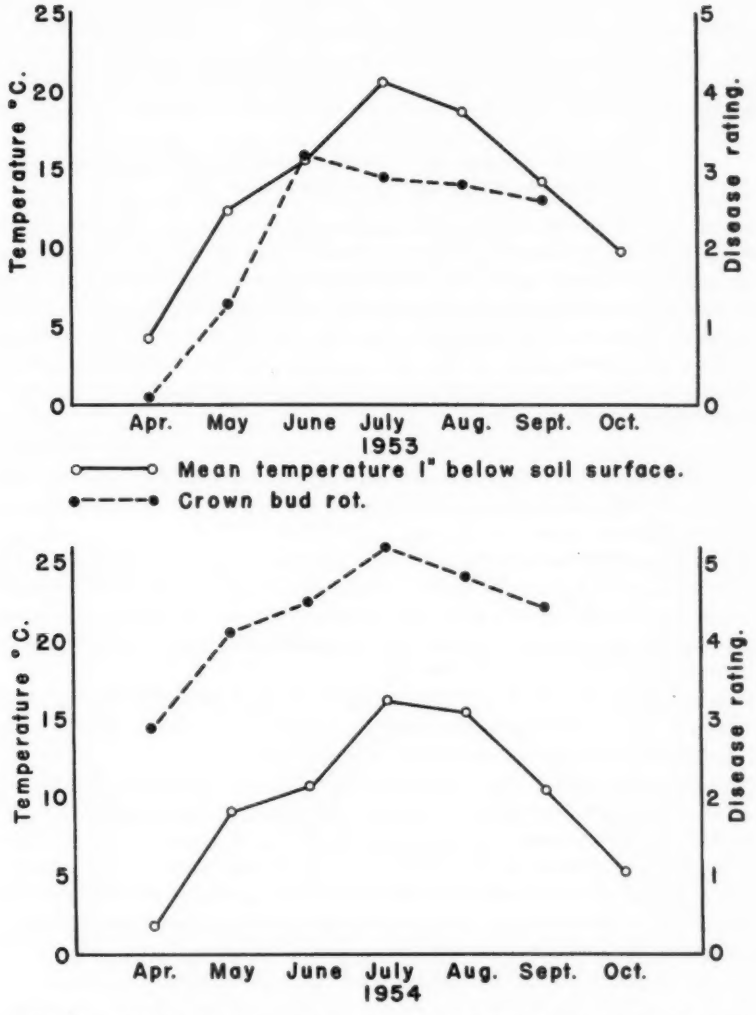

FIG. 9. Field plot development of crown bud rot of alfalfa in relation to soil temperature at crown level.

July. In both years crown bud rot development reached its peak during the month when the mean temperature of the soil in the vicinity of the alfalfa crown was 16° C. ±0.3° and the maximum damage took place when the soil temperatures were within the range of 9.0° to 13.0° C. The gradual climb in mean temperature to the high of 16.2° C. in 1954 was accompanied by an increase in crown bud rot whereas in 1953 temperatures in excess of 15.7° were not followed by further increases in the amount of disease.

State of Growth of Alfalfa Plants in Relation to the Development of Crown Bud Rot

In contrast to the development of winter crown rot of alfalfa (1, 5), crown bud rot built up most rapidy within a period of approximately one month in early spring (Table II) when the soil temperature was relatively low (Fig. 9). This rapid and early increase in the severity of the disease together with such phenomena as an almost complete absence of crown bud rot in first-year stands and the rapid development of the disease in the spring of subsequent years indicated that the state of growth of host plants was a factor of importance in the epidemiology of crown bud rot.

Young healthy alfalfa seedlings were transplanted to 6-in. pots of unsterilized soil in September. When the plants had become well established half of the pots were moved out of doors, buried to the level of the soil in the plot, and overwintered there.

These pots of alfalfa were brought into the greenhouse as soon as the frost had left the ground in early April. These plants and those that had been overwintered in the greenhouse were treated with a composite inoculum made up from soil cultures of the crown bud rot pathogens, incubated for 30 days, and then rated for crown bud rot.

Disease ratings, based on a total of 24 replicate pots of each treatment, were as follows: (*a*) greenhouse overwintered controls 2.1, inoculated 2.9; (*b*) out-of-doors overwintered controls 5.9, inoculated 8.4.

It would appear from these results that alfalfa crown buds were predisposed to crown bud rot by exposure to winter conditions. It is possible that the rate of disease development was governed largely by the amount of weathered and dead tissue present in the vicinity of the alfalfa crown. The average rating of 5.9 for the out-of-doors control series was probably the result of the growth of naturally occurring inoculum on this material when the plants were brought into the greenhouse in the spring.

The more positive results obtained when laboratory-grown inoculum was added (8.4) would seem to further substantiate the theory that the condition of the crown bud tissues was important.

Isolation Studies

During the years 1952 to 1955 isolations were made from bud-bearing sections of alfalfa crowns taken from each field sampled during the detailed survey.

Different methods of surface disinfecting plant materials and of isolating were tested (5, 7, 17, 18) to avoid as far as possible the differential selection of certain microorganisms. Satisfactory results were obtained by immersing alfalfa crown sections in a 1:1000 solution of mercuric chloride for 1½ minutes, removing the excess disinfectant with two successive washes in sterile water, and plating out on potato dextrose agar. Incubation was carried out at room temperature with final notes being taken after 12 to 14 days. Identification of the *Fusarium* species was based on the classifications of Gordon (10, 11) and Snyder and Hansen (21).

Fusarium species, especially *Fusarium avenaceum* (Fr.) Sacc. and *Fusarium acuminatum* Ell. & Ev., predominated among the isolates. For convenience and because of the difficulty of getting positive identification of all isolates taken, they were grouped under *F. roseum* Link sensu Snyder & Hansen and are listed as such in Table III. This group species was most prevalent regardless of sample time and it showed little seasonal variation in relative numbers.

Rhizoctonia solani Kühn ranked next in prevalence to *F. roseum*. Except for the fall of 1952 and the spring of 1954, most of these isolates appeared in summer samples.

The spring appearance of most of the *Ascochyta imperfecta* Peck isolates was to be expected in the light of the work reported by Cormack (3). This situation was true only in 1952 and 1953. The annual mean percentage of the total isolates of this fungus declined from the high of 9.8 in 1952 to 2.6 in 1955.

TABLE III

ANNUAL AND SEASONAL PREVALENCE OF PRINCIPAL ISOLATES TAKEN FROM THE CROWNS OF ALFALFA PLANTS SHOWING CROWN BUD ROT

Isolate	Survey season	% of total isolates				4-year mean
		1952	1953	1954	1955	
Fusarium roseum	Spring	37.7	59.4	30.6	54.0	45.5
	Summer	56.8	41.7	47.5	35.7	45.4
	Fall	44.4	41.6	51.0	59.9	49.2
	Mean	46.3	47.6	43.0	49.9	
Rhizoctonia solani	Spring	8.0	5.9	25.0	18.6	14.4
	Summer	26.9	41.1	31.0	30.2	32.3
	Fall	40.8	31.2	20.0	23.5	28.9
	Mean	25.2	26.1	25.3	24.1	
Ascochyta imperfecta	Spring	11.3	14.1	3.1	0.0	7.1
	Summer	8.9	1.6	2.2	7.2	5.0
	Fall	9.2	2.5	7.4	0.7	4.9
	Mean	9.8	6.1	4.2	2.6	

Succession of Crown Bud Rotting Fungi

Isolation data compiled from 1952 to 1955 inclusive were examined for evidence of fungus succession. Earlier work has shown that disease development was influenced by soil temperatures ambient to the alfalfa crowns. Now it seemed desirable to assess the effect of host plant age on the relative numbers of *F. roseum*, *R. solani*, and *A. imperfecta* isolates emerging from spring, summer, and fall survey specimens of alfalfa crowns. The results are given in Fig. 10.

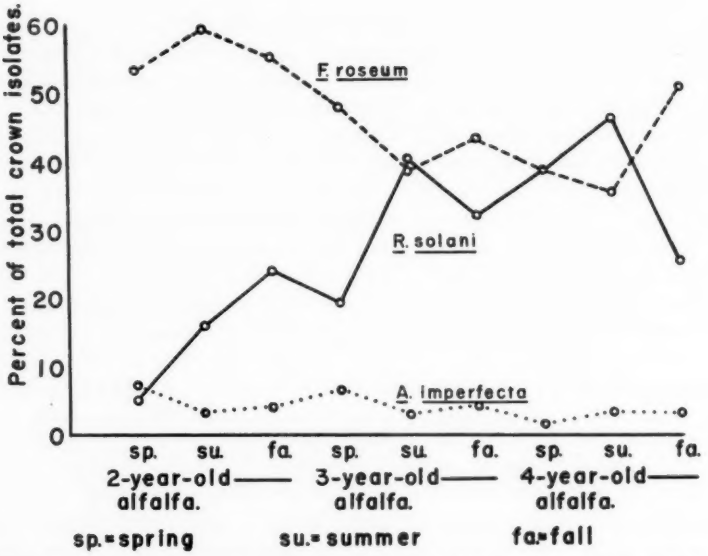

FIG. 10. Age of stand and season of the year in relation to the proportion of the principal fungi isolated from alfalfa crowns showing crown bud rot.

F. roseum isolates made up 55.9% of the total taken from second-year alfalfa crowns, with the number appearing in the summer samples being somewhat higher than at either of the other sampling times. *R. solani* isolates averaged 15.1% of the total for the year and *A. imperfecta* isolates comprised only 5.1% of the annual total.

Isolations made in third-year stands were considerably different. *F. roseum* and *R. solani* made up 43.4 and 30.7% respectively of the total isolates. For *R. solani* this represented an increase of slightly more than 100%. *A. imperfecta* did not show any marked deviation from that of the previous year.

The picture in fourth-year alfalfa showed definite indications of a change in relative numbers. Fall-sampled crowns yielded an increased number of saprophytic *Trichoderma* and *Penicillium* isolates and a marked decrease in the number of *R. solani*. There was a decided increase in the number of *F. roseum* isolates taken from the same material.

Infection Studies

Materials and Methods

Young alfalfa plants were transplanted into unsterilized 3:1 soil and sand (five plants per 6-in. pot) in the fall for use in these studies. As an alternative the test plants were often grown from seed and used after they were at least 3 months old. In the case of transplants they were allowed to become established in the soil before being inoculated.

Immediately before inoculation the top growth of the plants was cut back to approximately one inch to stimulate elongation and development of the existing buds and to initiate the formation of new crown buds.

Typical isolates of *F. avenaceum*, *F. acuminatum*, *R. solani*, and *A. imperfecta* were grown in a sterilized 1:1 mixture of soil and sand fortified by the addition of 10% (by volume) of corn meal. The surface of the soil in each 6-in. pot of alfalfa was evenly covered with a 50 ml. aliquot of inoculum. This was covered with a light layer of sieved soil, watered, and incubated at 23° C. ± 2°.

Results

After 15 days, representative material from each of the five treatments was photographed to show the effect on top growth (Fig. 8). All plants were then rated for crown bud rot. These data are given in Table IV.

TABLE IV

PATHOGENICITY TO ALFALFA OF *Fusarium acuminatum*, *Fusarium avenaceum*, *Rhizoctonia solani*, AND *Ascochyta imperfecta* ISOLATES TAKEN FROM ALFALFA PLANTS SHOWING CROWN BUD ROT

Test organism	Average crown bud rot rating*	Increase over control
Control	0.3	–
F. acuminatum	1.5	1.2
F. avenaceum	1.7	1.4
R. solani	4.4	4.1
A. imperfecta	1.4	1.1

NOTE: L.S.D. at 1%, 0.5.
*Average of nine replicates.

These isolates of *F. avenaceum*, *F. acuminatum*, and *A. imperfecta* were significantly but not strikingly pathogenic to alfalfa crown buds under the conditions of the test. In contrast the disease rating of 4.4 for *R. solani* inoculated plants showed that 44% of the crown buds had been rotted by this organism.

Interaction of Crown Bud Rot Isolates

Two, and sometimes three, of the crown bud rotting fungi were frequently isolated from a single crown bud. *R. solani*, if present, invariably appeared first, followed by *F. roseum* and finally by *A. imperfecta*. None of the fungi

appeared to be markedly antagonistic to the other components of the disease-producing complex. This possibility was investigated in two experiments in which alfalfa plants, grown in the greenhouse from seed, were treated with soil-grown inoculum of crown bud rot isolates of *F. avenaceum*, *F. acuminatum*, *R. solani*, and *A. imperfecta* used singly and in all combinations. In all cases the amount of inoculum used was limited to 50 ml. per 6-in. pot. In cases where two or more components were used equal parts of each were applied so that the total volume came to 50 ml.

Crown bud rot readings were taken after 15 days' incubation at greenhouse temperature. The results of the two tests (Table V) showed that the fungi, despite some inconsistency, displayed the following order of decreasing pathogenicity: *R. solani*, *F. avenaceum*, *F. acuminatum*, *A. imperfecta*. There was evidence of synergistic activity among isolates of *F. avenaceum*, *F. acuminatum*, and *A. imperfecta* as indicated by pathogenicity although it was of a lower order than in combinations in which *R. solani* was present. This synergism was particularly evident in combinations involving *A. imperfecta*.

TABLE V

DISEASE DEVELOPMENT IN GRIMM ALFALFA EXPOSED TO VARIOUS COMBINATIONS OF CROWN BUD ROTTING FUNGI

Treatments	Average crown bud rot ratings*	
	Test No. 1	Test No. 2
Control	0.1	0.2
R. solani	3.9	5.3
R. solani and *F. acuminatum*	5.0	5.3
R. solani and *F. avenaceum*	3.9	5.8
R. solani and *A. imperfecta*	4.9	5.4
F. acuminatum	2.2	1.8
F. acuminatum and *F. avenaceum*	3.1	1.5
F. acuminatum and *A. imperfecta*	2.4	1.9
F. avenaceum	2.1	3.0
F. avenaceum and *A. imperfecta*	2.5	3.0
A. imperfecta	0.1	0.7
R. solani, *F. avenaceum*, and *F. acuminatum*	3.2	4.7
R. solani, *F. acuminatum*, *F. avenaceum*, and *A. imperfecta*	4.3	4.6
R. solani, *F. acuminatum*, and *A. imperfecta*	3.0	4.0
F. acuminatum, *F. avenaceum*, and *A. imperfecta*	1.7	2.4

*Average of five replicates.

Discussion

The acceleration in crown bud rot development in May or June of the second and following years of alfalfa growth under irrigation suggests the influence of environmental conditions of a relatively stable nature. Soil temperatures proximal to alfalfa crowns follow a trend rather closely correlated with the pattern of disease development (Fig. 9). Crown bud rot reached its annual peak in 1953 and 1954 when the mean soil temperatures were 15.7° C.

and 16.2° C. respectively. Higher temperatures appear to inhibit the causal organism while permitting sufficient production and growth of new crown buds to give the measure of partial recovery observed in fall-sampled alfalfa (Tables I and II).

The vigor of host plant growth is another factor worthy of consideration in relation to the regular pattern of crown bud rot development. The disease is of no consequence during the first year of growth but develops rapidly during subsequent years, particularly the second and third (Table I). Furthermore alfalfa plants which have undergone a dormant period are more susceptible than those of a comparable age which have not. The major portion of each year's disease development always occurs early in the growing season (Table II), suggesting a stage of maximum susceptibility to crown bud rot.

The apparent succession of causal organisms shown in Table III and Fig. 10 indicates both a soil temperature and a host plant effect. The over-all activity of the *F. roseum* group as reflected by isolate numbers was not appreciably altered (Table III) indicating a broad temperature range. The numbers of *R. solani* isolates showed an increase in summer-sampled crowns particularly in second- and third-year alfalfa. During the fourth year when average crown bud rot damage was 4.3 (Table I) and 100% of the plants were infected, *R. solani* activity decreased markedly coincident with an appearance of increased numbers of *Trichoderma* and *Penicillium* isolates. This may indicate the existence of increased antibiotic activity of the type described by Weindling (22, 23) and thus provide a partial explanation for the reduction in rate of crown bud rot development in fourth-year alfalfa.

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GENETICS AND PHYSIOLOGY OF THE XANTHOPHYLLOUS MUTANT OF THE TOMATO[1]

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Abstract

Xanthophyllous is a dominant yellow-leafed mutant; the genotype *Xa Xa* is lethal in either the seed or the very early seedling stage, while the genotype *Xa xa* is viable but reacts differently to light intensity than does the normal *xa xa* plant. The 1 : 2 : 1 ratio is never realized because less than half the lethals appear as seedlings. The 2 : 1 ratio is affected by germination so that under good conditions for germination a 2 : 1 ratio is obtained, but under poor conditions the ratio may approach 1 : 1. Maximum likelihood formulae are given for calculating linkage when one gene is a dominant with recessive lethal effects, and it is shown that the simple product moment method gives similar results for these data. *Xa* is in linkage group VII (chromosome 10), and its lethal effect disturbs the monogenic ratios of all genes in this group. The following crossover values between these genes and *Xa* are found: *H* 44.5%, *pe* 50%, *t* 35.5%, t^v 31.7%, and *ag* 46.5%.

Under standard growing conditions the pigment concentrations of xanthophyllous contrasted with green is: chlorophyll, 790 instead of 3240 μg./g.; xanthophyll, 54 instead of 211 μg./g.; and carotene, 60 instead of 130 μg./g. Both green and yellow plants, when grown under various intensities and spectral compositions of light, have different responses. Green plants make only limited response to changes in light intensity, whereas *Xa xa* plants become green and cannot be distinguished from *xa xa* plants. From 600 to 8000 ft-c. the *Xa xa* plants show a linear trend of decreasing pigment (increasing yellowness) with increasing light intensity. The hypothesis is advanced that *Xa xa* plants make as much pigment as do green ones, but a light-dependent reaction breaks down the pigments faster than they are being formed.

Introduction

While mutants have been used extensively in developing genetic theory, relatively little use has been made of them as tools for the study of physiological processes. In the tomato there are a large number of mutants which affect the chlorophyll, anthocyanin, and carotin systems, but little use has been made of any except the last (Jenkins *et al* (6)). There are 11 mutants which affect the chlorophyll system, namely: l_1, l_2, t^v, *dv*, *rv*, *ys*, *lg*, *au*, *yv*, *v*, and *Xa* (Butler (2), Rick and Butler (10)). These genes are genetically distinct and produce different phenotypes through various modifications of the tomato plant's normal chlorophyll system. The mutant *Xa* commended itself to our attention because of its striking response to light conditions. *Xa* plants, grown under field or greenhouse conditions in the summer, were bright yellow and grew very poorly. In the fall or spring, plants of the same genotype became light green instead of yellow, and grew faster and fruited better.

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Material and Methods

The xanthophyllic mutant first appeared as a chimera in the tomato field at Guelph in 1936. Seeds from the green shoots of this plant produced green seedlings only, whereas seeds from the variegated branches produced a mixture of green and yellow plants in various proportions but no variegated seedlings. The green seedlings all bred true, but none of the yellow seedlings would breed true; they gave a segregation of 528 yellow to 274 green as well as 32 plants which were a brighter yellow, with shorter hypocotyls, and narrower cotyledons, and which died within 2 weeks after emerging from the soil. These data indicated that xanthophyllic was a dominant with recessive lethal action, the three classes of segregants being: *Xa Xa* lethal, *Xa xa* yellow, *xa xa* green. In order to find the linkage relationship of this new mutant, it was crossed in 1937 with various genetic tester stocks, and further crosses were made in 1954. It was selections from these crosses which were used in the physiological studies.

For the genetic experiments the plants were grown in flats in the greenhouse at Guelph, all seedling characters were scored under greenhouse conditions, and limited F_2's were transplanted to the fields in order to score for mature plant and fruit characters. The main experiments on the effect of light quantity and quality were also performed in the greenhouse although corollary experiments were also run in growth chambers with controlled temperature, light, day-length, and humidity. The total amount of solar radiation falling on the plants in the greenhouse was recorded by a phototube type of integrating light recorder. Light intensities were also checked periodically with a Weston light meter. To change the quality of the light, cellophane tents were used to cover the plants. The cellophane used was: No. 3101 clear, red, tango, green, and blue. The percentage transmission of light in the range of the wave lengths from 380 to 700 mμ was taken by the use of a Beckman spectrophotometer, and the range and maximum transmission of each type of cellophane was obtained.

The solvent extract method was used to analyze the chlorophyll, carotene, and xanthophyll content of the leaves. Readings were taken of the prepared extracts by means of the Klett Summerson photoelectric colorimeter, and the recorded values were converted to mg. of pigment per ml. of solution per gram of fresh leaf sample. To record the difference in color of the green and mutant plants, and also between the mutant plants grown under different light conditions, the Hunter color difference meter was used.

Germination and Monogenic Segregation

The fact that the mutation shows up as a chimera in diploid tissue, and that green segregants always breed true while the yellow ones do not, indicates that this is a dominant mutation. The fact that yellow plants are of two types, those which die in the early seedling stage, and those which live and produce seed which again gives the three types of plants, suggests that the homozygote is lethal. We therefore expect a ratio of 1 lethal : 2 yellow : 1 green, but chi square tests indicate that this is not realized. There is

always an excess of greens and a deficiency of lethals. In order to obtain more information on this point and also to find at what stage in the life history of the seed this lethal effect is exerted, the following experiment was performed. Sixteen flats were filled from the same mixture of two parts loam to one part sharp sand, and then 10 rows of 20 seeds each were planted in each flat. The seeds all came from F_1 plants of the same cross but were harvested from different plants grown in different years. Consequently the seed was not of the same age or the same degree of maturity. Unfortunately the elementary precaution of mixing the seed to ensure uniformity was not performed so some heterogeneity was introduced. The flats were treated as identically as possible, and the germination and segregation recorded by row and by flat. No significant differences between the germination in the various rows were noted, but there was a significant difference between the germination in the various flats. The germination within the 16 flats was not normally distributed as would have been expected if each batch of 200 seeds had had a uniform probability of germinating, but instead occurred in three discrete groups of low, medium, and high germination. Accordingly, the data in Table I are tabulated under these three groups.

TABLE I

MONOGENIC SEGREGATION IN 16 FLATS, EACH CONTAINING 200 SEEDS

No. of flats	Average germination	Lethal *XaXa*		Yellow *Xaxa*		Green *xaxa*	
		Plants	% S	Plants	% S	Plants	% S
3	58.8%	56	9.3	161	26.8	136	22.6
6	77.3%	165	13.8	499	41.6	264	22.0
7	87.0%	227	16.3	642	45.9	349	24.9
16	78.1%	448	14.0	1302	40.7	749	23.4
Expected 1 : 2 : 1		624.8		1249.4		624.8	
Chi square		50.0		2.2		24.6	(P = .00001)
Expected 2 : 1				1367.3		683.7	
Chi square				3.1		6.2	(P = .001)
Expected 2 : 1 (87.0% group only)				661		330	
Chi square				.55		1.09	(P = .2)

NOTE: % S = that class as a percentage of the seeds sown.

It will be seen from Table I that the total 448 : 1302 : 749 does not fit a 1 : 2 : 1 ratio, the chi square of 76.8 being much too large. Furthermore, if the lethal class is omitted, the remaining classes do not fit a 2 : 1 ratio because of a deficiency in the yellow or heterozygous class. When a similar test is made on the numbers from the seven flats showing the best germination, the results fit a 2 : 1 ratio. The heterogeneity shown in this table can be analyzed in another way by examining the differences in the percentages of germination. When this is done, the green segregants are found to come from 22 to 24.9% of the seeds planted which is close to the expected 25%. In the group with low germination the percentage of green (*xa*) plants is a little

lower but it is not significantly so. Thus the green segregants are little affected by the adverse factors which caused the differences in germination between the three groups, and the over-all germination rate for *xa* plants is 93.1%. The yellow segregants accounted for 26.8, 41.6, and 45.9% of the seeds planted in the three germination groups. These percentages are significantly different and are closely related to the percentage germination of each group. In flats showing low germination the percentage of *Xa xa* plants does not resemble the expected 50%. The over-all germination for this group was 81.2%, which indicates that the seeds of this group are only 7/8 as efficient as are the seeds of the green segregants. The *Xa Xa* segregates show the greatest effect of adverse environment; the percentage of this type in the seven flats with the highest germination is almost double the percentage found in the flats with low germination. The over-all germination of this type of plant was 56% of the expected, and even under the best conditions it was only 65%.

From these data one draws the conclusions that the observed ratios will be dependent on the environment; the better the germination, the closer the ratio will approach the theoretical 1 : 2 : 1. Any factor such as age and maturity of seed, genetic background, depth of planting, moisture, and temperature, which affect germination, will affect the monogenic ratio. One dose of the *Xa* gene reduces the germination efficiency by about one-eighth while two doses reduce it by nearly one-half.

The Linkage Relationship of *Xa*

Xanthophyllic plants were crossed with plants containing mutant genes whose linkage relationships were known; then the *Xa xa* F_1 plants were selfed and produced the F_2 segregations which are listed in Table II. The genes used are fully described by Rick and Butler (10) and are merely listed here. They are dwarf (*d*), yellow flesh (*r*), brachytic plants (*br*), potato leaf (*c*), fasciated fruit (*f*), anthocyaninless (*a*), anthocyanin loser (*al*), non-hairy (*H*), uniform ripe fruit (*u*), tangerine orange fruit (*t*), virescent-t (t^v), sticky peel (*pe*), anthocyanin gainer (*ag*), and entire or broad leaf (*e*).

Columns 8 and 9 of Table II give the chi square tests for the monogenic ratios of both the xanthophyllic gene and the test gene (the other gene involved in the linkage test). The monogenic test for *xa* is calculated on the basis of 2 *Xa xa* plants : 1 *xa xa* plant; no *Xa Xa* plants are recorded in these segregations. Table II shows that three of the 16 chi squares for *Xa* are significant at $P = .05$ level, and that the cross with *H* is highly significant. In view of the effect of germination on these ratios, these departures can be ascribed to this cause, and we need not reject the 2 : 1 hypothesis. For instance, the monogenic ratio in the case of the largest departure is 889 : 593 instead of the expected 988 : 494, indicating a deficiency of over 99 xanthophyllic plants. The monogenic chi squares listed in the penultimate column of Table II indicate that five of these are also significant at the 5% point. The first of these is the cross involving potato leaf (*c*) where the recessives are 18 plants

TABLE II

LINKAGE TESTS WITH DIGENIC F_2 SEGREGATIONS

Test gene	Group	Phase	Observed segregations				Monogenic		Linkage
			a	*b*	*c*	*d*	χ^2_{xa}	χ^2_T	χ^2_L
d	I	c	2,419	876	1,355	395	4.16*	0.13	11.70*
		r	47	13	28	12	2.00	0.00	0.96
r	II	r	25	10	10	5	0.25	0.67	0.08
br	III	c	359	127	200	71	2.07	0.54	0.01
c	IV	c	200	51	93	22	0.60	4.99*	3.45
f	V	c	117	33	39	12	5.73*	0.73	0.12
a	V	c	183	77	94	31	0.13	1.93	1.09
al	VI	c	674	185	343	131	2.97	1.19	6.00*
H	VII	r	634	255	478	115	29.76*	0.00	18.56*
u	VII	r	19	10	13	0	.11	0.03	5.43*
t	VII	r	335	121	220	27	1.03	5.84*	22.20*
t^v	VII	c	2,401	520	836	650	0.30	5.64*	356.20*
pe	VII	c	409	205	280	99	0.89	4.61*	0.79
ag	VII	c	2,171	751	1,037	417	0.02	6.67*	4.56*
e	XI	c	499	151	237	90	0.01	0.06	2.14
		r	622	158	311	118	2.51	3.04	7.55*

NOTE: c = coupling; r = repulsion.
χ^2_{xa} (xanthophyllic) $= 1/2n\ (a + b - 2c - 2d)^2$.
χ^2_T (test gene) $= 1/3n\ (a - 3b + c - 3d)^2$.
χ^2_L (linkage) $= 1/6n\ (a - 3b - 2c + 6d)^2$.

less than expected. Since this character has been widely used and generally gives good ratios, this small departure will be classified as a chance one. The other four significant chi squares all occur in linkage group VII, and since it will be shown later that *Xa* also belongs to this group, these departures from good monogenic ratios are the result of the lethal *Xa Xa* carrying a disproportionate number of the linked genes with it.

Turning to the last column of Table II, we find that half the tests indicate association or linkage, and since the independence of these groups has been established by both linkage and trisomic data, some explanation other than simple linkage will have to be found for some of these cases. The first case is a coupling cross involving dwarf (*d*) in which there is a deficiency (395 instead of 420) in the double recessive class. If we assume that 1750 is correct for *xa xa* plants, then there are 205 fewer *Xa xa* plants than expected, and applying the same reasoning to the dwarfs we find that there are 86 more *Xa xa dd* plants than expected. Putting all this information together, we arrive at the following explanation. Germination difficulties have reduced the *Xa xa* tall class by some 205 plants; therefore if these are added to this class the ratio should have been:

Observed	2624	876	1355	395
Theoretical (6:2:3:1).....	2625	875	1313	437

which gives a good fit for the three classes but still leaves the double recessive

class significantly short. The only explanation we can offer for this is misclassification of some 30 dwarf plants which may occasionally happen in small seedlings; at any rate it is in the wrong direction for linkage.

The second significant linkage is with *al* of the VI linkage group. In this case the monogenic ratios are in order and the germination disturbance is slight. The product moment method of calculation gives a crossover value of 45.2%. The last line in Table II lists the segregation of *Xa* with broad leaf (*e*). Inspection of this ratio shows that the association revealed by the chi square is in the opposite direction from that expected in a repulsion cross, so the data are not consistent with the hypothesis of genetic linkage.

Turning next to the six tests with linkage group VII genes, we find that five of these show chi square values for linkage which are significant. In most of these cases the chi square for the tester gene also indicates disturbance of these ratios, so we have the problem of estimating the linkage values in a disturbed segregation and then correcting the monogenic ratios for the effect of a lethal gene with this linkage intensity. Bailey (1) has shown that the product moment method is fairly efficient even with disturbed ratios so, because of the ease of calculation by the use of Stevens (11) tables, this method will be used for the preliminary estimate. To check this estimate and also for the calculation of the theoretical ratios, the following equations are used:

	Coupling		Repulsion	
	m	$1/m(dm/dp)$	m	$1/m(dm/dp)$
Xa T	$\frac{1}{2}(1 - p + p^2)$	$2p - 1/1 - p + p^2$	Same as for coupling	
Xa t	$\frac{1}{2}(p - p^2)$	$1 - 2p/p - p^2$	Same as for coupling	
xa T	$\frac{1}{4}(2p - p^2)$	$2 - 2p/2p - p^2$	$\frac{1}{4}(1 - p^2)$	$-2p/1-p^2$
xa t	$\frac{1}{4}(1 - p)^2$	$-2/1 - p$	$\frac{1}{4}p^2$	$2/p$

It will be seen from this that the first two segregate classes have the same values for both coupling and repulsion, the values in the third and the last columns can be used as score values (Fisher (4)) for estimating, by the method of maximum likelihood, the p or recombination values.

Using the segregation with *H* we get 42.9% for the crossover value by the product moment method and 44.8% by the method of maximum likelihood. Using the 44.8% value and adjusting the observed for the deficiency of *Xa*, we get the following observed and theoretical values:

Observed (adjusted).....	856	329	478	115
Theoretical.....	892	293	473	120

The adjustment of the data makes no difference to the linkage estimate by the maximum likelihood method, but does increase the product moment estimate from 42.9% to 43.4%.

In the case of uniform (*u*), linkage is again indicated but the numbers are too small to make a valid estimate of linkage. The chi square for the sticky peel gene (*pe*), which is also in group VII, does not indicate linkage, so we can assume that the locus of this gene is well over 50 units from *Xa*. With the absence of linkage, there is no logical explanation for the disturbed monogenic segregation.

The crossover values for the *Xa t* section are 35.3% by the product moment and 35.5% by the score method. This linkage value would give a ratio of 361 : 108 : 205 : 29, which gives 137 tangerine segregates instead of the 176 expected on the basis of the 3 : 1 ratio. Actually we observed 148 in this class, and therefore the chi square of 5.84 indicates a shortage which is the result of the lethal action of *Xa*. The gene virescent tangerine (t^v) came from J. W. Lesley, who indicated that it was a multiple allele of *t*. It is characterized by the proximal parts of the leaves near the growing tip being yellow, and the distal parts green. Some of the *Xa* t^v plants can be picked out in the seedling stage by the different shades of yellow on the proximal and distal parts of the leaf, but in order to be certain of the classification it is necessary to grow the plants to flowering, at which time the orange color of the staminal cone characterizes a *t* or t^v plant, thus making it unnecessary to wait for the ripe fruit color. The *Xa* t^v cross is in the coupling phase; the crossover value by product moment is 33% and by maximum likelihood, 31.7%. These values compare quite favorably with those for *Xa t*, and since these are based on greater numbers, and are in the coupling stage with its smaller errors, this crossover estimate has greater accuracy. It is interesting to note that in this case the monogenic ratio of t^v is disturbed by an excess of 43 *Xa* t^v plants, indicating that the lethal effect of *Xa* has reduced the T^v segregates more than it has the t^v ones.

The segregation for *ag* indicates crossovers of 48% and 46.5% by the two methods. The monogenic ratio for *ag* is disturbed by the excess of 74 *Xa ag* plants, most of which can be accounted for by the lethal action of *Xa*. The observed and expected classes based on 46.5% crossover are:

	Xa Xa Ag	*Xa Xa ag*	*Xa xa Ag*	*Xa xa ag*	*xa xa Ag*	*xa xa ag*	TOTAL
Expected	1145	315	2191	725	1042	417	5834
Observed	lethal	lethal	2171	751	1037	417	4376

The crossover values given above, in conjunction with the other values given by Rick and Butler (10), would indicate a linear order and distances between marked loci of: *pe* 36 *u* 24 *H* 22 *t* 32 *Xa*. The gene *ag* is 46.5 from *Xa* according to this study, which would place it either between *u* and *H* or else on the other side of *Xa*. The segregations for *ag* with *H* and t^v are:

ag H	901	282	329	154	crossover 44.5%
ag t^v	1141	440	450	77	crossover 39.0%

This would place *ag* as distal to *Xa* but the crossover values are not all consistent.

The significant χ^2 for linkage between *Xa* and *e* in repulsion can probably be dismissed as a chance one since the coupling cross shows no such linkage.

Thus we conclude that *Xa* is in linkage group VII, which trisomic tests have shown to be on chromosome 10. It is closest to the *t* locus, and the order *pe, u, H, t, Xa* is consistent although some of the crossover values are out of proportion. The location of *ag* is in doubt, and must await further backcrossing. The *Xa al* linkage must also await further clarification.

The errors of the crossover values are not given because recent heterogeneity studies by the senior author have indicated that in the tomato the crossover values are not the same in pollen formation as in the ovule formation, and that they are probably affected by temperature and soil acidity. Until these points are clarified, the errors have little meaning.

The Physiology of Plastid Pigments in Xanthophyllic Tomatoes

This mutant was selected for genetic studies because it segregated so clearly into the three color classes. It was called "xanthophyllic" because this name connoted a yellow plant as compared with normal green ones, and also utilized "*x*", one of the least used of the letters which symbolize the specific genes of the tomato. At the time of naming, no thought was given to a chemical analysis of pigments; thus the name was not intended to infer that this mutant had a higher concentration of xanthophyll. At the onset of this study it was important to get some quantitative method of characterizing the greenness or yellowness of a plant. The simplest method is by means of the Hunter color difference meter. Readings from the meter for plants taken in mid-summer were:

Green plants	*Rd*, 6.6	*a*, −11.5	*b*, 12.3
Yellow plants	*Rd*, 13.9	*a*, −13.4	*b*, 19.0

It will be seen that the greatest difference is in the *Rd* or color values; the chroma or *a* value is indicative of a yellow-green color, with green increasing as one moves to the negative end of the scale. The *b* or hue value in this region of the scale is constructed in such a way that the higher the positive value of *b* the more yellow the hue.

By chemical analysis the amount of the various pigments in 1 g. of leaf was obtained. This gave pigment concentrations:

	Chlorophyll	Xanthophyll	Carotene
Green plant	3240 μg./g.	211 μg./g.	130 μg./g.
Yellow plant	790 μg./g.	54 μg./g.	60 μg./g.

This gives a ratio of 25 : 1.6 : 1 for the green plants as opposed to 14 : 1 : 1 for the yellow plants, therefore there is nearly twice as much chlorophyll per unit of xanthophyll or carotene in the green plants as there is in the yellow ones. The analysis also shows that there is almost four times as much of these pigments in a green plant as there is in a yellow one.

Since these standards have been established and since it has been indicated that the values may be light-dependent, it appears advisable to limit comparisons to values from the same stock grown at the same time under similar conditions. Accordingly, each experiment will be analyzed separately. When material was grown in the winter under adverse light conditions, it was found that the separation of green from yellow plants was almost impossible, so an experiment was set up in the summertime using three sets of 250 F_2 seeds, each set being subjected to a different light history. Set No. 1 was kept in the greenhouse under normal daylight and thus received a 12–15 hour exposure

TABLE III

HUNTER COLOR DIFFERENCE READINGS FOR F_2 SEGREGANTS UNDER DIFFERENT LIGHT CONDITIONS

Set No.	Light	Plant	*Rd*	*a*	*b*
Two weeks after germination					
1	Normal	Green	7.9	−11.7	13.1
1	Normal	Yellow	14.9	−13.4	19.0
2	500–600 ft-c.	Green*	5.3	−10.0	10.1
3	500–600 ft-c.	Green*	6.0	−10.7	12.2
Four weeks after germination					
1	Normal	Green	8.0	−10.5	12.6
1	Normal	Yellow	13.2	−13.8	19.5
2	500–600 ft-c.	Green*	7.1	−11.0	11.8
3	2 weeks of normal	Green	8.1	−12.8	14.4
3	2 weeks of normal	Yellow	13.4	−14.0	20.7

*The two classes of segregants are indistinguishable, all are green.

which varied in intensity from 1000 to 8000 ft-c. Set No. 2 was placed in a light chamber and subjected to 500–600 ft-c. for 14 hours each day. Set No. 3 was placed in the same chamber for 2 weeks and then exposed to normal sunlight. The results are given in Table III.

These data show that under low light intensity the readings of both yellow and green segregants are identical, and that these readings are similar to those obtained for green plants under normal light conditions. The readings at 4 weeks after germination are extremely revealing because in set No. 2, which has been kept under low light intensity, the *Xa* and *xa* plants are indistinguishable and give green type readings, whereas in set No. 3, which has had 2 weeks of low intensity followed by 2 weeks of normal light, the green/yellow segregation is apparent. Furthermore, the readings for these segregants are similar to the readings for yellow and green plants which have spent all 4 weeks under normal light. This experiment shows that the gene *Xa* cannot produce its phenotypic effect when grown under light intensities of 600 ft-c. or less.

It is apparent that light intensity has a differential effect on *Xa* and *xa* segregants, but it is also interesting to know whether this effect is caused by all wave lengths or else just certain ones. Accordingly, two experiments were set up in which the plants were grown under cellophane tents which transmitted light of different wave lengths (Fig. 1). Unfortunately this simple method of controlling wave lengths of transmitted light also changed the intensity, but it was hoped that, if these results were of sufficient interest, plant physiologists with more elaborate equipment would carry them out in greater refinement. One experiment was done in a growth chamber under controlled light intensity and day length, and the other was carried out in the greenhouse.

In the growth chamber with 13 hours of daylight at 1600 ft-c., the chlorophyll content of the green plants was not significantly different under any of the

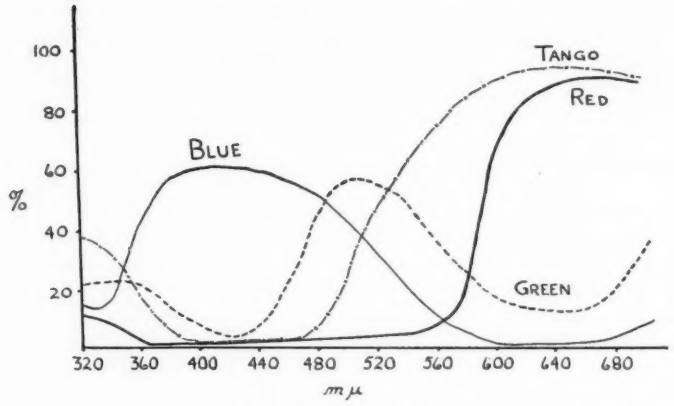

FIG. 1. The percentage of light of different wave lengths transmitted by the colored cellophanes.

treatments although the plants grown under the tangerine cellophane had 2980 mg./g. of pigment, which is higher than the average of 2820 mg./g. The yellow plants, on the other hand, do show significant differences between treatments as well as between the successive samples which were taken at 2-week intervals. The statistical analysis of the data of the chlorophyll content gave the following results:

SOURCE OF VARIANCE	S.S.	D.F.	S^2	F	F_{01}
Total	2.4971	23			
Between treatments	1.2130	5	.2426	9.74**	4.56
Between dates	.9112	3	.3037	12.19**	5.42
Error	.3729	15	.0249		

It will be seen from this analysis of variance that the differences both between treatments and between dates are highly significant. Since light and other environmental conditions were kept constant, the differences between dates must be the result of something intrinsic to the plants. Close examination of the means for different dates shows that there is no linear relation between the four dates. The August 30 analysis, which was made when the plants had been only 2 weeks in the growth chamber, shows an extreme increase over both succeeding and preceding measurements, while the three dates September 15, 29, and October 13 show a gentle upward slope from the low value of September 15. Since the sudden increase on August 30 is probably the result of different factors from the gentle increase of the other three dates, a new analysis was performed omitting the August 30 analysis. This gave:

SOURCE OF VARIANCE	S.S.	D.F.	S^2	F	F_{01}
Total	.6280	17			
Between treatments	.5064	5	.1013	17.17**	5.64
Between dates	.0630	2	.0315	5.34*	7.56
Error	.0586	10	.0059		

It will be seen that this analysis brings about a great decrease in the total variance, and that the difference between dates is still significant at the 5% point but not at the 1% point. This difference between dates can be accounted for either by adaptation of the plants to this environment, or to the reduced growth causing a greater concentration of pigment-bearing cells in the sample. Since in these three dates the trend effect is small and is represented to approximately the same extent in each treatment, it will be ignored. Using the means for each treatment and the light values in foot-candles transmitted, we get the linear equation $Y = 1.110 - 0.000256\,X$, where Y is mg./g. of chlorophyll and X is light intensity in foot-candles. When this is plotted it shows a good fit for the control, clear, tango, and blue but not for green and red. The green has 0.16 mg./g. less than expected and the red has 0.17 mg./g. more than expected if light intensity alone were the critical factor, so quality of light is also important. If we plot the data for August 30, we find that it fits into the same pattern; a line of the same slope fits these data but the intercept is 0.35 mg./g. higher. As was the case with the other set of data, the red and green do not conform. Plants raised under red have almost twice (0.8 mg./g. more) as much chlorophyll as would be expected if light intensity alone were the causal factor, while plants raised under the green cellophane have 0.25 mg./g. less chlorophyll than expected.

To find out whether the quality of light was the critical factor, the abscissal values were changed from transmitted light to relative effective energy for chlorophyll production by the use of the values obtained by Frank (5). The use of the relative effective energy values alone, or of these values multiplied by light transmitted, was found to give a poorer fit than the use of the light transmitted values alone. Therefore neither quality of light nor the relative effective energy explain the discrepancy in the chlorophyll content of *Xa* plants grown under green and red cellophane. A possible explanation for this is that the *Xa* plants have a different effective spectrum for chlorophyll formation and that we are not justified in using the one derived from green plants of different species.

In the greenhouse the light intensity was not constant during the growth period from July 7 to October 1, the average daily total calories per cm.^{2} for each period preceding the analyses being:

July 7—Aug. 5	595	Sept. 3—Sept. 16	466
Aug. 6—Aug. 12	571	Sept. 17—Oct. 1	331
Aug. 13—Sept. 2	400		

The main comparison between green and yellow plants was made on Sept. 16 and Oct. 1, and the data are presented in Table IV. During this period the light intensity fell from 650 to less than 500 g-cal./cm.^{2}/24 hours. Samples taken from near the growing tips on the two dates gave the following values of mg./g. of chlorophyll:

	September 16	October 1
Green plant	3.5 ± .14	3.5 ± .07
Yellow plant	1.15 ± .15	.87 ± .10

TABLE IV

PIGMENT CONSTITUTIONS OF *Xa xa* AND *xa xa* PLANTS GROWN IN THE GREENHOUSE UNDER COLORED CELLOPHANES

Treatment	Date	Chlorophyll, mg./g.		Carotene, μg./g.		Xanthophyll, μg./g.	
		Xa xa	*xa xa*	*Xa xa*	*xa xa*	*Xa xa*	*xa xa*
Clear cellophane	Sept. 16	0.811	3.511	67.36	109.54	67.04	130.4
	Oct. 1	0.520	3.032	43.34	100.70	53.58	113.95
Red cellophane	Sept. 16	1.444	3.790	70.74	117.31	59.20	93.06
	Oct. 1	1.129	2.896	63.91	93.60	51.55	92.40
Tango cellophane	Sept. 16	1.091	4.159	73.53	104.65	82.06	166.21
	Oct. 1	1.040	3.376	52.28	82.54	74.31	117.15
Green cellophane	Sept. 16	1.616	3.303	54.36	105.56	51.94	124.50
	Oct. 1	0.942	3.076	55.42	106.99	45.64	148.92
Blue cellophane	Sept. 16	1.456	3.348	76.16	105.56	80.59	131.78
	Oct. 1	1.031	3.046	64.42	107.50	62.90	137.03
Control	Sept. 16	0.483	3.384	48.52	123.38	73.22	144.37
	Oct. 1	0.559	2.889	47.55	107.94	52.64	108.88

The over-all picture does not give enough detail since we are particularly interested in the differential effects of the various colored cellophanes; therefore, each color must be considered separately. When this is done we find that analyses of green plants grown under clear, green, and blue cellophane are not significantly different from the control, and that these can be grouped together for both dates. Plants grown under the red tent had a higher chlorophyll content on September 16 but this fell to the level of the rest by October 1. The green plants grown under tango-colored cellophane had a significantly higher chlorophyll content on both dates, the values being:

	September 16	October 1
Control, clear, Blue, green	3.39 ± .04	3.01 ± .04
Red	3.79	2.90
Tango	4.16	3.38

Thus, under fall greenhouse conditions, the green plants did not react to the modification of the light spectrum brought about by the use of clear, green, or blue cellophane, but they reacted to red cellophane by an increase in chlorophyll content at first, followed by a return to the basic concentration. The reaction to tango was definite and lasting; the content had increased by 23% on September 16 and 12% on October 1.

The yellow plants, on the other hand, showed a different reaction to the colored cellophanes, the chlorophyll content showing an inverse relation to the light intensity. The straight line relationship between the chlorophyll content and light intensity was $Y = 1.814 - 0.0167\ X$ which means that a

decrease of 4000 ft-c. (control to tango) brought about an increase of 0.67 mg./g. in the chlorophyll content. This is strikingly shown in the increase in the chlorophyll content of the yellow plant under the clear plastic as compared with the uncovered control as shown in Table IV. The October 1 analyses show a similar but less pronounced trend with the equation $Y = 1.231 - 0.0091\ X$. Thus the presence of the *Xa* gene, when contrasted with its *xa* allele, caused the chlorophyll production mechanism to respond differently when exposed to light of different intensities and wave lengths.

The main differential responses took place in the controls and in the plants under green and blue. The green plant controls show a decrease in chlorophyll content between September 16 and October 1, while the xanthophyllous controls show no change. Green plants grown under green and blue cellophane have the same chlorophyll content as the controls, whereas xanthophyllous plants grown under green and blue show an increased content of chlorophyll.

Since the main differences found are in the xanthophyllous plants, the data for the *Xa xa* plants on the five dates are treated in more detail in the analysis of the chlorophyll results given below:

SOURCE OF VARIANCE	S.S.	D.F.	S^2	F	F_{01}
Total	10.417	59			
Between treatments	3.593	5	.7186	10.21**	3.41
Between dates	3.303	4	.8257	11.73**	3.72
Error	3.521	50	.0704		

It is obvious that the variability due to dates cannot be ignored and since we cannot be sure that the treatments did not act differently on different dates it is best to seek material that is relatively homogeneous for dates. Inspection of data (Chang (3)) indicates that the chlorophyll content is lowest on August 5 and highest on August 12 and that the September 2, 16, and 30 analyses are relatively constant. Accordingly we can pool these three dates and get rid of the difference between dates of sampling as shown in the following analysis:

SOURCE OF VARIANCE	S.S.	D.F.	S^2	F	F_{01}
Total	3.784	35			
Treatment	2.790	5	.558	16.91**	3.76
Dates	.062	2	.031		
Error	.932	28	.038		

Examination of the treatment means in the data for these last three dates of analysis shows that again they are ranked according to the amount of light transmitted with the possible exception of red. The value of 1.179 for red is lower than the expected of 1.4 and it is interesting to note in this connection that the August 12 value for red was 2.342. In Fig. 2 the values for control, red, and blue are plotted against the dates of analysis and this shows that the control stays more or less uniform, whereas the chlorophyll content of plants grown under red cellophane increases greatly in the first 2 weeks and then falls to a steady value for the next month. Blue, on the other hand, shows a less spectacular increase at 2 weeks, and then, unlike red, it again increases for the next 2 weeks.

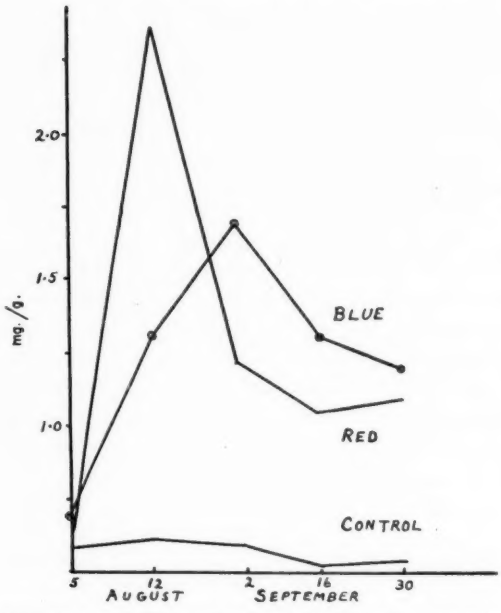

FIG. 2. The chlorophyll content of xanthophyllic plants grown under colored cellophanes.

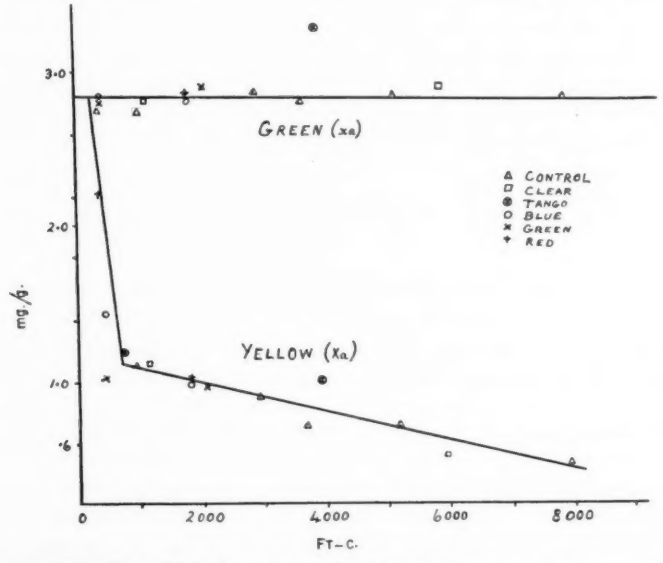

FIG. 3. The effect of light intensity on the chlorophyll content of green and xanthophyllic plants.

The general picture of the behavior of the concentration of chlorophyll in yellow and green plants under different light intensities is shown in Fig. 3, where the mean concentrations from all experiments are plotted against light intensity. This graph shows that the chlorophyll content of the green plants does not change significantly when the light intensity is increased from 450 ft-c. to 8000 ft-c. The only exceptions are the plants grown under the tango cellophane. These plants have a higher chlorophyll content than their counterparts which were grown under the other colors. The yellow plants give a vastly different picture. Decreasing the light intensity increases the concentration of chlorophyll. Between 600 and 8000 ft-c. there is a uniform linear increase, but below 600 ft-c. there is a rapid doubling of the pigment concentration, and this may be independent of light intensity. Thus there appear to be two different phases with 600 ft-c. as the dividing point. Observational evidence also indicates that the pigment increase which takes place at low light intensities is confined to the growing tip and to any growth which has never been exposed to light intensities greater than 600 ft-c. On the other hand, the pigment increase which takes place at the low light intensities is lost within 5 days of exposure to higher light intensity, and the green leaves become yellow.

The carotene pigment is 45.8 $\pm$ 5.4 mg./g. higher in the green plants than it is in the yellow ones. The quality of light does affect the pigment concentration in the green plants, but the results are not easily explained on the basis of light intensity alone as can be seen by an inspection of Table IV. On both dates the control has the highest concentration of carotene but by October 1, concomitant with the decreasing light intensity, there is a 15 mg. drop in pigment. On September 16 the plants under the red cellophane had the second highest content of carotene but by October 1 they had dropped to the second lowest, with only tango showing a lower value. The xanthophyllous plants behave differently in three different experiments but the control always has the lowest concentration of carotene. This is probably the result of the increased light intensity of the control as compared to any of the treatments. The highest concentrations are in the plants grown under the red and the blue, and these results again fit into the light intensity pattern. The main exception to using light intensity as the causal factor is the plant under green cellophane which has 20 mg. less than expected. Thus the carotene concentration can be ranked from highest to lowest as follows:

Green plants—control, clear, blue, green, red, tango
Yellow plants—blue, red, tango, green, clear, control

The xanthophyll content of the green (*xa*) plants is twice as high as that of the yellow (*Xa*) plants (Table IV). Ranking the treatments of the two phenotypes in order of decreasing pigment concentration we get:

Green (*xa*) plants—tango, green, blue, control, clear, red
Yellow (*Xa*) plants—tango, blue, control, clear, red, green

It is at once apparent that the concentration of xanthophyll is not dependent on the light intensity since in both phenotypes the ranked order places the control and clear (highest light intensities) in the middle of the ranks instead of at one end. It is interesting to note that tango gives the highest values for both phenotypes. In green plants the effect of blue and green cellophane is to increase the pigment concentration, whereas in yellow plants, only blue produces this increase. Since this is an important point, the data from Chang (3) are examined and it is found that green cellophane does produce an increase in this pigment in some cases, but that the increase is not as marked as that brought about by the blue cellophane. The method of chemical analysis employed gives the least reliable measurements for xanthophyll, so these findings can only be accepted as preliminary.

As is expected (Rabinowitch (9)), the growing of plants under the conditions of these experiments brings about structural alterations in the leaf. Leaves were embedded in paraffin, sections cut and stained, and then measurements were made of leaf thickness, cell size, and plastid size and number. These results are not reported in detail because differences in growth rate make standardization of sections difficult under our experimental conditions, and also the results are similar to those already published for other plants. Most of these alterations are what would be expected from the changes in light intensity; decreasing the light makes the leaves thinner, the cells smaller, and reduces the number of chloroplasts. The effects of blue and green cellophane are not compatible with a linear effect of light intensity. In these two treatments the cell size is slightly larger than expected, while the leaf thickness and chloroplast number are much larger than expected. This is especially so in plants grown under the green cellophane. In view of the vastly different light transmitted by these colored cellophanes (Fig. 1), it is impossible to ascribe the structural modifications to any one cause.

The foregoing results are similar to those of Koski and Smith (8), who postulate that the mutant white seedling-3 differs from normal corn plants in its inability to retain chlorophyll, and that the pigment deficiency is not the result of a "block" of some step in the biosynthesis of chlorophyll, but rather the result of the abnormal rate of breakdown of the pigment. The behavior of the yellow (*Xa*) plants fits into a similar hypothesis. Plants with the genotype *Xa xa* apparently make as much chlorophyll as the normal *xa xa* plants, but at light intensities greater than 600 ft-c. the chlorophyll in the *Xa xa* plants is broken down faster than it is being built up, and therefore the plant becomes yellow. This reaction is not completely reversible since leaves which have become yellow do not become green when the light intensity is decreased. The "greening up" of a yellow (*Xa xa*) plant takes place chiefly by the production of new growth. Koski *et al.* (7) with corn, and we with tomato, found no differences between the plastids of normal and mutant seedlings, whereas Schwartz (12) found that a close correlation exists between plastid size and chlorophyll development in white, virescent, and pale-green mutants in corn.

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NOTES ON NEARCTIC HEPATICAE

XIII. THE GENUS TRITOMARIA (LOPHOZIACEAE) IN ARCTIC CANADA[1]

RUDOLF M. SCHUSTER

Abstract

Only four species of *Tritomaria* Schiffn. have been described, all found in cold to boreal regions of the northern hemisphere. Of these, only *T. quinquedentata* (Huds.) Buch has been known from the arctic portions of eastern Canada. The range of this species in Canada east of the 100 meridian is tabulated, and two varieties, var. *turgida* (Lindb.) Weim. and var. *grandiretis* Buch and Arnell, are described from the same area. The latter variant is here first recorded from North America: it is presumably a polyploid, possessing larger cells and more numerous oil-bodies. *T. heterophylla* Schuster is described from materials from northernmost Ellesmere Island. It is allied to *T. scitula*, from which it differs in purplish pigmentation, broader than long leaves, and spinose-dentate perichaetial bracts. The last peculiarity serves to differentiate it from all other species of the genus. A key to all of the species and varieties, all known from eastern Canada, is given.

Introduction

The family Lophoziaceae is the largest family of Hepaticae in the temperate and colder portions of the northern hemisphere; it is also by far the most difficult to treat systematically. In recent years the writer (19, 20, 22, 23, 24) has devoted much effort to this polymorphous group. The genus *Tritomaria* was treated at some length in two contributions (20, 23) based wholly on a study of non-arctic materials.

During the year 1955, the writer spent between 3 and 4 months doing field work in the high Arctic of northeastern Ellesmere Island, between latitudes 82° and 83° North and longitudes 62° and 66° West, some 500 miles south of the North Pole. Three entities belonging to the genus were found, two of which were new to North America (and had not been previously treated). One of these taxa is apparently identical with *T. quinquedentata* var. *grandiretis* Buch and Arnell, a putative polyploid derivative of *T. quinquedentata*, previously known from northernmost Sweden and Siberia. The other taxon is typical *T. quinquedentata*, while the third represents a sharply defined new species. A key differentiating these three entities, as well as the others previously reported from North America, follows.

ARTIFICIAL KEY TO SPECIES

1. Mature leaves 0.75–1.0× as wide as long; always with reddish-brown gemmae abundantly developed; cells of leaf-middle from 8–10 × 10–16 μ up to 17–22 μ wide × 20–30 (36) μ. ..... 2.
2. Mature leaves strongly asymmetrically trilobed, the antical lobe a mere sharp tooth situated *ca.* midway between base and apex of leaf; postical leaf margin strongly arched and much longer than the nearly straight antical margin. Only on acidic substrates ..... 3.

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Contribution from the Department of Botany, University of Massachusetts, Amherst, Mass.

3. Cells of leaf-middle 8–12 μ wide × 10–18 μ long, generally with small trigones (and often somewhat thick-walled, the lumen becoming guttulate); cells of middle of postical margin 8–12 × 10–15 μ; gemmae elliptical, smooth, 8–12 × 11–18 μ; perianth-mouth with rather numerous, crowded cilia, mostly 1–(2) cells wide at base ..... *T. exsecta*

3. Cells of leaf-middle 17–22 μ wide × 20–36 μ long, usually with bulging trigones, the intervening walls thin; cells of middle of postical margin 18–20 μ; gemmae angulate, 14–18 × 17–26 μ; perianth-mouth with more distant cilia, often 2 to several cells broad at base..... *T. exsectiformis*

2. Mature leaves subequally trilobed, the antical lobe virtually as well developed as the other two, postical leaf-margin hardly more distinctly arched than antical, subequal to it in length or barely shorter; cells and gemmae as in *T. exsectiformis*. Only on basic or subbasic substrates..... *T. scitula*

1. Mature leaves 1.15–1.45× as wide as long; gemmae angulate; cells of leaf-middle 21–25 (25–32) μ wide × 24–30 (30–42) μ long, little elongated; trigones large, bulging; sinuses between lobes obtuse to rectangulate, subequal, shallow..... 4.

4. Leaves subequally trilobed, the antical lobe (and margin) almost as long as the postical; stem postically, and the postical leaf-bases, vineous pigmented (only the distal portions of the leaves purplish- to yellowish-brown); leaves below gynoecia, and bracts, sharply dentate to spinose-dentate; cells with extremely large trigones, often subconfluent, thus quite pellucid; subapical cells 21–25 × 24–30 μ, virtually equal in size to median cells; gemmae always freely developed, reddish-brown, the larger 27–35 μ long; plant small, 1–1.5 mm. wide, with facies of *T. scitula*, often in part with 2-lobed leaves..... *T. heterophylla*

4. Leaves quite asymmetrically trilobed, the antical lobe (and margin) markedly shorter than postical; stem (and sometimes leaves) postically clear brown pigmented, the leaves distally often yellowish-brown in color, never with any trace of vineous; leaves below gynoecia, and bracts, with lobes entire-margined or merely sinuate-dentate; gemmae very rare, yellowish to pale brown; plant robust, 2–3.6 mm. wide usually, never with simply bilobed leaves..... *T. quinquedentata*—5.

5. Marginal cells of lobes (15) 17–21 (23) μ; apical and subapical cells of lobes 16–21 (23) × 18–23 μ; median cells (18) 21–24 × 25–35 μ; oil-bodies mostly 3–7 (8-10) per cell, in the subapical and median cells..... 6.

6. Leaf-lobes sharply cuspidate, ovate; plants yellowish-green to somewhat yellowish-brown, at most the postical leaf-bases slightly brownish-pigmented; cells often slightly smaller, exceptionally with very coarse trigones; not in bogs; never with facies of *Saccobasis*..... *T. quinquedentata* typical

6. Leaf-lobes short and broad, often obtuse or subobtuse, not or hardly cuspidate, the postical lobe often rounded; usually more or less brownish; cells slightly larger, often approaching those of the next in size, often with very coarse trigones; bog and swamp plant; often like *Saccobasis*..... *T. quinquedentata* var. *turgida*

5. Marginal cells of the lobes 23–28 (30–35) μ; apical and subapical cells, in lobes, (21) 23–28 (30) × 24–35 μ; median cells (23) 25–32 × 30–42 μ; oil-bodies 9–17 (18–19) per median cell; cells with very coarse, bulging trigones..... *T. quinquedentata* var. *grandiretis* Buch and Arnell

The present paper is restricted to a study of the two arctic species, *T. quinquedentata* and its varieties, and to the newly discovered *T. heterophylla*. The discovery of the latter, however, has certain implications which deserve special consideration here. *T. heterophylla* has the broader-than-long leaves of *T. quinquedentata*. However, it approaches *T. scitula* (see Schuster (23), Pl. 25, Figs. 7, 10 *e*, 12) in the subequally trilobed form of the leaves, and in the frequently bilobed leaves. It furthermore agrees with *T. scitula* in the form of the perianth-mouth, in the ubiquitously produced gemmae, and in facies. This species, therefore, serves in some way to connect *T. quinquedentata* (assigned by Schuster (20, 23) to a special subgenus, *Trilophozia* Schuster) and *T. scitula* (which has been placed, with the other species of the genus, in the typical subgenus *Eutritomaria*). The discovery of this species is therefore of

more than ordinary interest, in view of the fact that the position of *T. quinquedentata* in *Tritomaria* does not stand without challenge. Meylan (13) believed it belonged better in *Barbilophozia* (or in *Lophozia*, if *Barbilophozia* is considered to be a mere subgenus of *Lophozia*). Müller (15) still states that "über die Richtigkeit der Einreihung dieser Art bei *Tritomaria* kann man im Zweifel sein. Sie weicht von *T. exsecta* ab durch zweireihigen Antheridienstiel (wie bei *Barbilophozia*), durch 5-schichtige Kapselwand, anderen Stengelquerschnitt mit zwei Reihen kleinerer, derbwandiger Rindenzellen und doppelt so grossen Markzellen (i.e., wie bei *Barbilophozia*), durch so breite wie lange Blattform (bei *exsecta* oval)." Of the objections raised by Müller to the inclusion of *T. quinquedentata* in *Tritomaria*, that dealing with leaf form no longer exists. The difference in the antheridial stalk is, in my estimate, nearly meaningless. Müller (14) certainly strongly overevaluated the systematic importance of this structure, particularly in old and plastic families like the Lophoziaceae and Lepidoziaceae. In *Lophozia excisa*, for example, I have been able to show that the stalk may be uniformly 2-seriate, uniformly 1-seriate, or partly 2-, partly 1-seriate on individual plants. Müller (15) again regarded the biseriate antheridial stalk of the Lepidoziaceae as a "family characteristic," although Schuster and Blomquist (25) soon showed that in the lepidozioid genera *Telaranea* and *Microlepidozia* it is 1-seriate. In *Lophozia* subgenus *Massula* Müller (15) admits of the existence of both 1- and 2-seriate antheridial stalks. As a consequence, this cannot serve as a real criterion in deciding whether *T. quinquedentata* belongs in *Tritomaria* or not. Similarly, Müller states that in *T. exsecta* the capsule wall is 3-stratose and, in *T. scitula*, 4-stratose. The 5-stratose capsule wall of *T. quinquedentata* is correlated with significantly greater robustness of both gametophyte and sporophyte, compared to these two species, and probably deserves to be interpreted as a purely functional difference without systematic value. The 2-stratose cortex of *T. quinquedentata* is perhaps of greater systematic significance. However, it surely does not deserve consideration, per se, as warranting removal of *T. quinquedentata* from *Tritomaria*. Of much greater significance, the dorsiventral differentiation of the medulla, emphasized by Buch (3) as a generic character of *Tritomaria*, clearly occurs in *T. quinquedentata*, although the mycorrhizal, small-celled "ventral strand" is often less strongly marked than in such typical species of *Tritomaria* as *T. scitula* (compare, for instance, Plates 25, Figs. 1 and 27, Fig. 5, in Schuster (23)). Such dorsiventral medullary differentiation, with development of a small-celled mycorrhizal ventral band, is not or is scarcely indicated in *Barbilophozia*.

The writer would conclude, therefore, that the position of *T. quinquedentata* in *Tritomaria* may be regarded as positively established. Indeed, the two subgenera *Trilophozia* and *Eutritomaria*, previously used to emphasize the isolated position occupied by *T. quinquedentata* as opposed to the other species of the genus, may well be reduced to the rank of mere sections.

Descriptions

1. Tritomaria heterophylla sp. n.

Similis *T. scitulae* facie; plantae fuscentes, bases foliarum saepius purpurascentes; foliae multo latiores quam longae, subaequaliter trilobatae (rarae foliae bilobatae); cellulae lobarum foliarum 21–25 × 24–30 μ; corpora oleifera magna, 2–7 per cellulam; gemmae communes, rufo-fuscentes ad purpurascentes; bractae feminae et foliae desuper spinoso-dentatae in margine; ostia perianthi crenulata ad setulosa, dentibus rariter ad duas cellulas longitudinae. Cetera desunt.

Similar in facies to T. scitula, the plants forming small, *prostrate*, ± *brownish or purplish-brown patches*, only the apices of the shoots ascending, unless strongly crowded, when suberect. Shoots simple or rarely terminally furcate (supporting leaf of branch bilobed, lacking the postical lobe), 1000–1350 (1650) wide × 8–16 mm. long, the distal portions of the leaves *pellucid and pale green* (shade forms) or brownish, the leaf-margins sometimes decolorate distally, the *postical leaf-bases slightly to prominently purplish pigmented*, the *distal portions of the leaves often also with a rosy hue*, the underside of the *stem* ± *purplish-black* pigmented. Stems quite fleshy, straight and rather rigid, 275–300 (380) μ in diameter; dorsal cortical cells markedly elongated, *ca.* 19–22 μ wide × (60) 75–100 μ long, their longitudinal walls strongly thickened (*length–width ratio ca. 3–5:1*). Leaves weakly to moderately imbricate, stiffly and obliquely laterally spreading, often somewhat complicate (especially when bilobed), virtually transverse, the *larger or uppermost somewhat* fluted, *transversely oblong*, ranging from 820 μ wide × 700 μ long (1.17× as wide as long) to 950 μ wide × 720 μ long (1.32× as wide as long) up to 1050–1200 μ wide × 750–820 (900) μ long (to 1.40–1.46× as wide as long), *subequally trilobed* for 0.2–0.3 their length (*occasional leaves subequally bilobed*); lobes ovate-triangular, acute to subobtuse, *usually distinctly apiculate, on upper leaves often sparingly spinose-dentate*, often with slightly reflexed margins, on upper leaves of gemmiparous shoots occasionally with *scattered, sharp to aciculate teeth*; sinuses obtuse to somewhat acute, *narrowly but sharply reflexed and gibbous* at base. Cells *very strongly collenchymatous*, the bulging trigones sometimes subconfluent, on margins of lobes 20–25 (27) μ tangentially measured; subapical cells and those of middle of lobe 21–25 μ wide × 24–30 μ long, *hardly smaller than median cells*, which are (22) 23–27 × 25–30 (35) μ; basal cells 23–27 μ × 30–38 μ; cuticle weakly verruculose to striolate; oil-bodies spherical to ellipsoidal, 2–7 (8) per cell in leaf-lobes, (2) 3–10 per cell in median cells, from 5 to 6 μ up to 6 × 9, occasionally 7 × 13 μ, finely papillose-segmented; chloroplasts *ca.* 3–3.5 μ. Gemmae 1–2-celled, in reddish-brown to purplish masses at apices of juvenile leaves, from 18 to 21 × 28 μ to 23 × 24–27 μ *up to a maximum of 24–32* × *35* μ.

Dioecious. Male plants unknown. Female plants with leaves gradually larger above, *their margins with scattered spinose teeth*. Bracts exceedingly polymorphous, varying from obtrapezoidal to transversely oblong, larger than leaves, varying from 1350 μ wide × 1000 μ long to 1700 μ wide × 1300 μ long

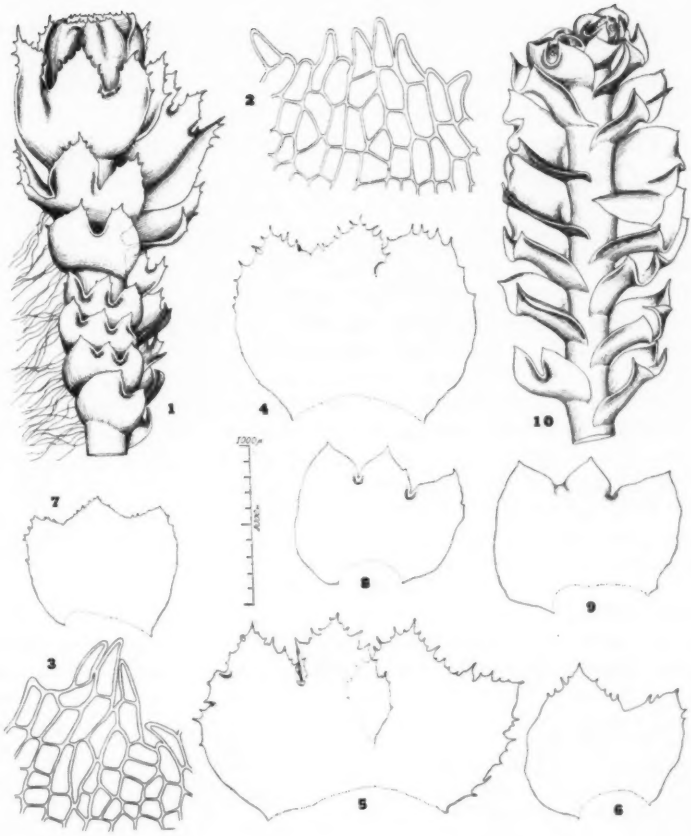

FIG. 1. *Tritomarla heterophylla* Schuster. 1. Shoot with perianth, unfertilized plant. 2–3. Portions of perianth-mouth. 4. Female bract. 5. Female bract. 6. Leaf below gynoecium. 7. Gemmiparous leaf. 8–9. Sterile leaves. 10. Sterile shoot. (Figs. 1, 10, *ca.* 18×; Figs. 2–3 drawn to same scale as cell-drawings in Fig. 2. Figs. 4–9 drawn to same scale, 25×, to scale at left of Fig. 8: figures all from *Schuster 35103*).

(*width 1.3–1.35* × *length*), up to 1900 μ wide × 1150 μ long (*ca.* 1.6–1.7 as wide as long), erect or suberect (at least when perianth is immature), *strongly plicate and undulate-crispate*, (2) 3–4 (5) lobed, to 1/4 to 1/3 their length, the sinus narrow at base, *strongly reflexed to gibbous*; lobes ovate-triangular, their *margins, and margins of bracts for some distance below the lobes, sharply spinose-dentate to ciliate-dentate*, and ending in similar spinose teeth, the teeth commonly formed of 2–3 superimposed slender cells (the terminal 15–18 × 45–64 μ). Perianth (immature only known) with mouth crenulate-dentate to

crenulate-setulose, bearing tapering, narrow teeth (formed of cells *ca.* 12–13 × 30–48 μ long), which are either free for their distal halves, or frequently wholly free, occasionally with scattered 2-celled teeth (of which the terminal is slender and acuminate).

Type

Foot of Mt. Pullen, on northeast slope, *ca.* 5 miles S. of Alert, NE. Ellesmere Island, *ca.* 82° 30′ Lat. N. (*R. M. Schuster 35103*).

Isotypes

On NW. slope of "The Dean," about one mile east from Mt. Pullen (*R. M. Schuster 35102, 35120, 35156a, p.p.;* with *Cryptocolea imbricata, Odontoschisma macounii, Tritomaria quinquedentata, Blepharostoma trichophyllum brevirete, Gymnomitrium concinnatum*, etc.); exposed, thin, peaty soil over basic scree slope, E. side of Mt. Pullen (*R.M. Schuster 35315a, 35307c, p.p., 35133b, p.p.*, with *Lophozia heterocolpa* var. *harpanthoides, Blepharostoma trichophyllum brevirete, Tritomaria quinquedentata, Cephaloziella arctica, Cryptocolea imbricata, Lophozia quadriloba, Odontoschisma macounii, Solenostoma polaris, Scapania gymnostomophila*, etc.); exposed soil-covered, damp, snow-fed scree slope, 2000–2400 ft., east edge of the United States range, 9–10 miles due west of Mt. Olga, *ca.* 82° 24′ N., 65° 20–30′ W., NE. Ellesmere Island (*R.M. Schuster 35615a, p.p.*; Aug. 7, 1955; with *Anthelia juratzkana, Scapania polaris*, etc.).

Distribution

Known only from the type collection and a series of isotypes from the same general area; probably a high-arctic derivative of the low-arctic *T. scitula*.

Ecology

The type specimens were found on gentle slopes, over weakly calcareous, moss-covered rubble and weathered rock, moistened by the runoff from persisting snow banks, at an elevation of *ca.* 450–600 ft., in full sun. Associated were *Lophozia heterocolpa, L. quadriloba, L. alpestris* var. *major, Blepharostoma trichophyllum grandirete, Anthelia juratzkana, Cephaloziella arctica, Tritomaria quinquedentata, Scapania* sp., *Arnellia fennica*, and *Plagiochila arctica*.

The species occurs on humus lying over calcareous shale and clay; in its ecology evidently it is very close to *T. scitula*.

The isotypes, from "The Dean," only about one mile away, occurred over peaty, somewhat acid humus over basic and subbasic rocks, at the mouth of a shallow ravine, at the head of which was an extensive persisting snow bank, resulting in rather constantly moist conditions. Associated were all of the preceding species of Hepaticae, as well as *Cryptocolea imbricata, Gymnomitrium concinnatum*, and *Odontoschisma macounii*.

The species appears confined to locally moist situations, in full sun (during the very short arctic summer), contiguous to snow banks which "feed" into the moss–herb tundra lying below it. Although evidently associated with

calcareous rock outcrops, the species undergoes ecesis usually only *after* a rather distinct humus layer is deposited. In this respect, the species is very similar to *Odontoschisma macounii*.

Associated vascular plants are *Saxifraga caespitosa*, *S. oppositifolia*, *S. flagellaris*, *Papaver radicatum*, *Salix arctica*, *Cerastium alpinum*, etc.

Differentiation

A strongly isolated species, recognizable in the field (with hand-lens) on the basis of the somewhat crispate, trilobed, broad leaves, the often somewhat pellucid appearance of the leaves (more so than in *Tritomaria quinquedentata*, which is a relatively opaque plant), and by the vineous pigmentation of the leaf-bases. The vineous pigmentation of the leaf-bases usually extends to a greater or lesser extent to the rest of the leaves, which may be somewhat rosy in color, although the purplish pigmentation may be largely hidden by a brownish secondary pigment. The plant is most distinctive when the female plants are evident. These latter bear somewhat larger leaves distally, imbricate, strongly crisped and undulate, the margins of whose lobes are quite evidently spinose-dentate to spinose-ciliate. No other known species of *Tritomaria* has such distinctive bracts, so highly differentiated from vegetative leaves. The species also differs from all other species of *Tritomaria*, except *T. quinquedentata*, in the broad, transversely rectangulate leaves, ranging from about 1.15–1.45× as broad as long (measured to apex of ventral lobe, which is slightly the longest in most cases).

The type and isotype material was collected, from a number of closely contiguous sites, always admixed with *T. quinquedentata*. The two species were at once habitually distinguishable, differing in a large number of features, among them: (*a*) plants only 1–1.25, rarely to 1.5 mm. wide in *T. heterophylla*, (1.5) 2–3.5 mm. wide in *T. quinquedentata*; (*b*) plants somewhat pellucid in appearance in *T. heterophylla*, quite opaque in *T. quinquedentata*; (*c*) trigones and subapical cells distinctly larger in *T. heterophylla* than in *T. quinquedentata*; (*d*) the ventral stem surface, and postical leaf-bases, to at least a slight extent, vineous or purplish pigmented in *T. heterophylla*; in *T. quinquedentata* at least the stems brownish postically with age, the ventral leaf-bases in extreme cases dark chestnut-brown pigmented, without trace of any reddish color complement; (*e*) leaves approaching a symmetrical form in *T. heterophylla*, strongly asymmetric in *T. quinquedentata*; (*f*) dorsal cortical cells 19–22 μ wide in *T. heterophylla*, 14–17 μ wide in *T. quinquedentata*; (*g*) gemmae always present in *T. heterophylla*, normally lacking in *T. quinquedentata*. In addition to these differences, *T. heterophylla* differed from the xeromorphic phase of *T. quinquedentata* present at the type station in the spreading and loosely canaliculate leaves (vs. concave and often suberect leaves in *T. quinquedentata*), giving the two species a distinct facies. Most of these distinctions are clear from Figs. 2 and 3, drawn from intermingled plants of the two species.

In several of the above features, particularly (*a*), (*e*), and (*g*), *T. scitula* closely agrees with *T. heterophylla*. Furthermore, both species grow closely

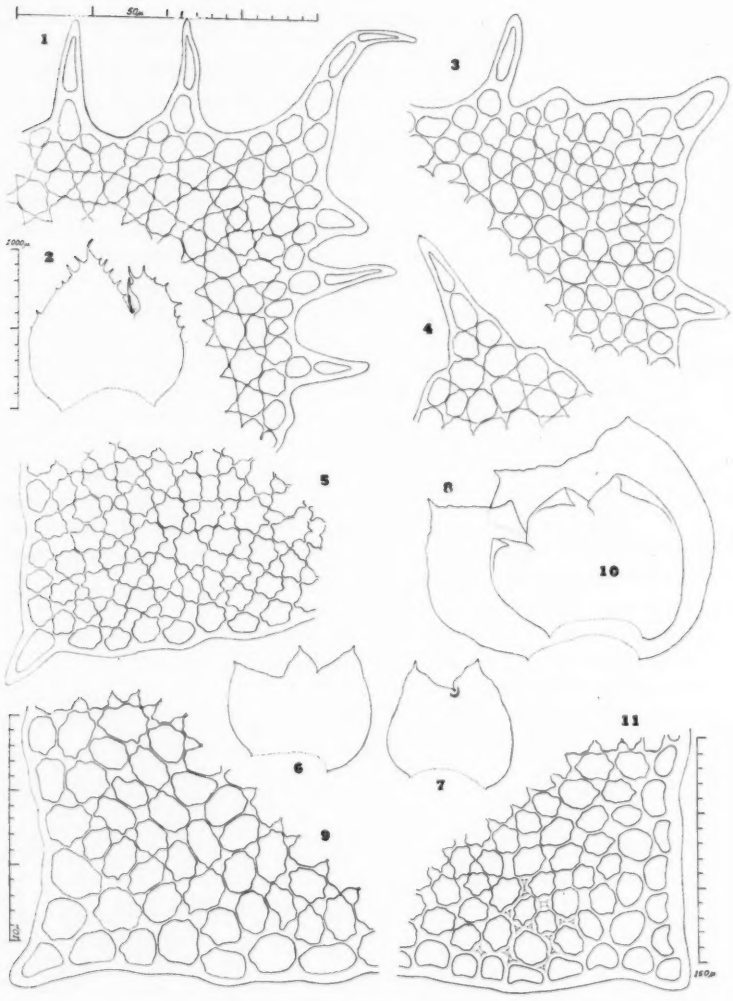

FIG. 2. *Tritomaria heterophylla* Schuster. 1. Lobe of female bract. 2. Entire female bract. 3–4. Apices of vegetative leaves of female plant. 5. Lobe of sterile plant. 6–7. Two leaves from sterile plant. *Tritomaria quinquedentata* var. *grandiretis* Buch et Arnell. 8. Leaf. 9. Cells of apex of middle leaf lobe. *T. quinquedentata* (typical). 10. Leaf. 11. Apex of middle lobe of leaf. (All cell drawings to same scale, ×235, drawn to scale indicated at top and bottom of figure; leaf drawings all to one scale, ×25, drawn to scale indicated at left margin of figure. FIGS. 1–7 from *Schuster 35103*; 8–11 from *Schuster 35593*.)

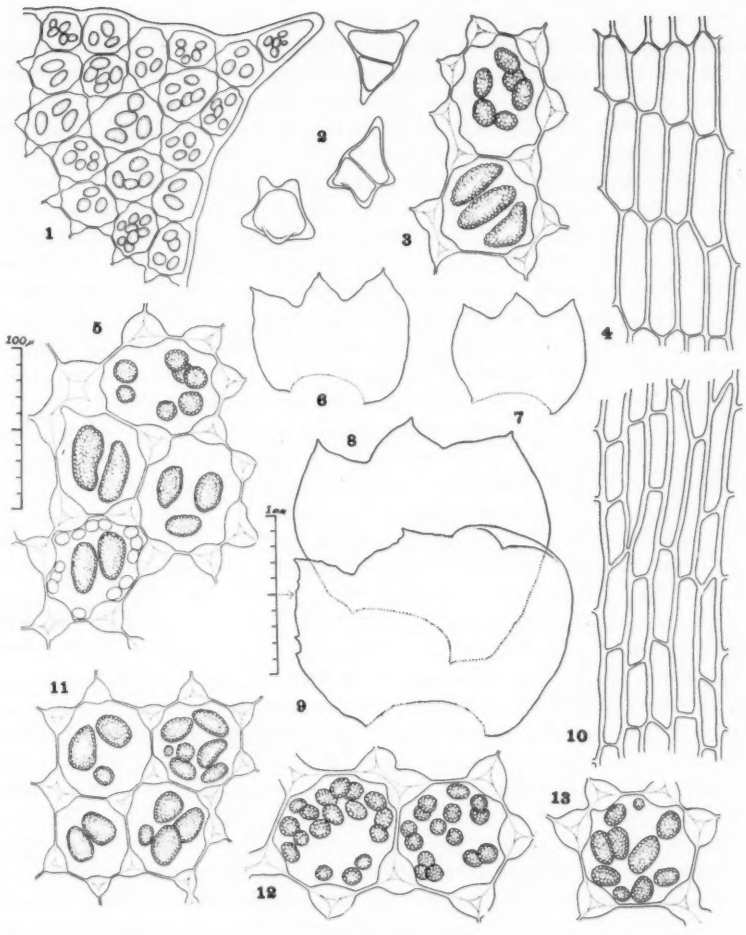

FIG. 3. *Tritomaria heterophylla* Schuster. 1. Cells of lobe, with oil-bodies drawn in. 2. Gemmae. 3. Median cells with oil-bodies. 4. Dorsal cortical cells of stem. 5. Cells of subapical part of ventral lobe of leaf. 6–7. Two sterile leaves. *T. quinquedentata* (Huds.) Buch, typical. 8. Leaf. 9. Leaf. 10. Dorsal cortical stem cells. 11. Subapical cells of lobe. *T. quinquedentata grandiretis* Buch et Arnell. 12–13. Subapical cells with oil-bodies drawn in. (Fig. 1, ×345; 2, ×450; 3, 5, 11, 13, ×645; 4, ×250 (scale to left of Fig. 5); 6–9, ×25, drawn to scale at left of Fig. 9; 10, ×300. Figs. 1–7 from *Schuster 35102*; 8, 10–11, from *Schuster 35102a*, admixed with *35102*; 9, 12–13, from *Schuster 35593*).

adnate to the substrate, unless crowded, both often produce isolated bilobed leaves, and both bear gemmae in abundance. However, the gemmae of *T. heterophylla* are often more sharply stellate and up to 35 μ in longer diameter; the leaves of gemmiparous plants occasionally or often bear sharply spinose, scattered teeth; and the leaves are always markedly broader than long, the inverse situation prevailing in all forms of *T. scitula*. In addition to these differences, there is the marked difference in facies, due to the tendency towards production of purple pigmentation of the postical leaf-bases in *T. heterophylla*, a pigmentation pattern which does not recur in any of our other species of the genus. In this, *T. heterophylla* closely approaches the allied genus *Saccobasis*, which it further resembles in the subequally trilobed leaves. However, the leaves in *T. heterophylla* are much broader, and the cells somewhat smaller, and less elongated in the leaf-bases, than in *Saccobasis*. The leaf-insertion in *T. heterophylla* is also of the usual *Tritomaria* type, and therefore much less complex than in *Saccobasis*.

In spite of the marked differences in leaf-shape, in pigmentation, and in the size of the gemmae, *T. heterophylla* is perhaps best regarded as a high-arctic derivative of *T. scitula*. When material with inflorescences is available, *T. heterophylla* is sharply separable from *T. scitula* (as well as all other species of the genus) by the form of the female bracts and subinvolucral leaves. Both the bracts and leaves subtending them have the lobes more or less cuspidate or spinose-dentate at the apex, and bear scattered sharp, spinose to spinose-ciliate teeth. The perianth-mouth is distinct from that of *T. exsecta*, *exsectiformis*, and *quinquedentata* in being very short dentate, with the teeth mostly 1–2-celled. In this respect, *T. heterophylla* again approaches *T. scitula*.

A possible relationship may also occur with the arctic *T. quinquedentata* var. *turgida* (Lindb.) Weim., described from the Jenissei River of Siberia. Persson (17) reports this variety from Alaska and Jensen (9) reported it from Hurry Inlet, on the east coast of Greenland (a reference that Persson (17) appears to have overlooked). It was also reported from Ellesmere Island by Bryhn (2). According to Persson, it occurs rather commonly in northern Scandinavia. This variety differs from typical *T. quinquedentata*, according to Persson, in the "leaf lobes (which are) shorter and broader, little or not cuspidate, often obtuse or nearly so . . ." In the leaf-lobes it differs even more markedly from *T. heterophylla*, which often has cuspidate or mucronate leaf-lobes. Müller (15) also mentions this variety of *T. quinquedentata*, stating "Eine grosse in Sümpfen der Nordländer lebende Form (var. *turgida* Lindb.) besitzt am hinteren Blattlappen keine Stachelspitze." The var. *turgida*, however, is "often more or less brownish" but never appears to have the purplish pigmentation of *T. heterophylla*.

2. *Tritomaria quinquedentata* (Huds.) Buch
Jungermannia quinquedentata Hudson, Fl. Angl., Ed. 1, 511, 1762.
Jungermannia barbata var. *quinquedentata* Nees, Natur. eur. Leberm. 2, 196, 1836.

Jungermannia lyoni Taylor, Trans. Bot. Soc. Edinburgh, 1, 116, pl. 7, 1844.
Lophozia quinquedentata Cogn., Bull. Soc. Bot. Belgique, 10, 279, 1872.
Lophozia lyoni Steph., Spec. Hep. 2, 149, 1902; Evans, Rhodora, 4, 210, 1902.
Barbilophozia quinquedentata Loeske, Verh. Bot. Ver. Brandenburg, 49, 37, 1907.
Lophozia (Subgenus *Barbilophozia*) *quinquedentata* K. Müller, Rabenh. Krypt.-Fl. 6 (1), 624, Fig. 298, 1910; Meylan, Beitr. Kryptogamenfl. Schweiz 6 (1), 160, Fig. 96, 1924; Macvicar, Studs. Hdb. Brit. Hep. Ed. 2, 193, Figs. 1–4, 1926.
Lophozia asymmetrica Horikawa, J. Sci. Hiroshima Univ. Ser. B, Div. 2 (2), 153, Fig. 16, 1934.
Tritomaria quinquedentata Buch, Mem. Soc. F. Fl. Fennica, 8 (1932):290, 1933; K. Müller, Rabenh. Krypt.-Fl. Ed. 3, 6 (1), 736, Fig. 240, 1954.
Tritomaria (subgenus *Trilophozia*) *quinquedentata* Schuster, Am. Midl. Nat. 45 (1), 65, Pl. 24, 1951; Schuster, Am. Midl. Nat. 49 (2): 382, Pl. 27, 1953.

Plants *prostrate*, in often extensive patches or mats, pure green to yellowish-brown or pale brown; *shoots creeping*, ascending at the very apex (unless crowded), robust, 2–3 (3.6–4) mm. wide × 1.5–5 (6) cm. long, remotely branching. Stems subterete, green to somewhat brownish, deep brown or purplish-brown ventrally (due to mycorrhizal infection), 350–450 μ in diameter, *ca.* 18–22 cells high; *cortical cells in 2–3 layers*, thick-walled and of smaller diameter, *ca.* (10) 14–17 (20) μ, than medullary cells, subisodiametric in cross section, varying from 35–45 μ long up to (38) 45–72 (80) μ long (length:width *ca.* (2) 3–6:1); medullary cells in upper 0.6–0.8 of stem rather large and pellucid, thin-walled and with small trigones, *ca.* 22–36 μ in diameter, the *lower 5–8 layers of medullary* cells much smaller in diameter, *ca.* 18–24 μ (nearly similar to cortical cells), thin-walled and with small trigones, becoming *mycorrhizal with age and brownish*. Rhizoids long and dense, forming a conspicuous ventral mat. Leaves weakly to distinctly imbricate, the *antical end transversely inserted*, the postical two-thirds inserted *ca.* 36–45° obliquely (from horizontal), not decurrent, *usually horizontally and widely spreading*, but (at least when crowded) *sometimes with antical lobe loosely folded over the larger postical "half,"* the leaf then loosely complicate-canaliculate, *strongly asymmetric, orbicular-ovate* to subreniform, *averaging wider than long* (*ca.* 1.2–1.6 mm. wide × 1–1.4 mm. long), rarely as wide as long (1020 × 1020 μ), often *somewhat fluted, strongly unequally trilobate* (the ventral lobe longest, to *ca.* 1300–1400 μ; median lobe *ca.* 1100–1200 μ; antical lobe *ca.* 1000–1050 μ in length × 1500–1600 μ in total leaf width), the *lobes broadly ovate-triangular*, spreading (or the *postical often incurved*), *apiculate to cuspidate* from an obtuse base; sinuses descending 0.1–0.25 the leaf-length, *broadly obtuse or occasionally* crescentic, often somewhat gibbose, *those between antical and median lobes narrower or subequal to those between median and postical lobes*. Cells strongly collenchymatous, thin-walled and with distinct to bulging trigones, of leaf-margins and leaf-apices *ca.* (*16*) *18–23* μ, subisodiametric; median cells *ca.*

(18) 20–24 × 21–28 (30) μ, at leaf-base *ca.* 23–28 × 28–38 μ; cuticle weakly verruculose; *oil-bodies usually 2–7 (9–10) per cell*, spherical (and 4.5–6 μ) to ovoid or ellipsoid (and 3.5 × 5.5 to 4.5 × 8 to 7 × 8–10 μ), finely granular-papillate; chloroplasts *ca.* 3–3.5 μ. *Asexual reproduction rare*, via small groups of *yellowish to yellowish-brown 1–2 celled gemmae* at apices of the uppermost leaves; gemmae tetrahedral to polyhedral, 15–20 μ.

Dioecious, but often with sex organs. Androecial plants usually in separate patches; androecia loosely spicate, the bracts in 4–12 (rarely 16–20) pairs, more transversely oriented than leaves, somewhat similar to them in shape and size, but strongly ventricose at base, the antical (and sometimes median) lobes erect or suberect, sharp; antheridia 2–3, their stalk 2-seriate; a few linear to lanceolate paraphyses usually present. Gynoecia with bracts broader and slightly longer than leaves, erect with spreading lobes, 3–5-lobed to 1/4 to 1/3, plicate or crispate, the *lobes rarely with obscure accessory teeth;* bracteole small or obsolete to rather large, when distinct at all fused for much of its length with bracts. Perianth 0.65–0.75 emergent, cylindrical-obovoid, 2–3-stratose at least in basal fourth, pluriplicate above middle, rounded and narrowed to mouth; mouth lobulate, the lobes with polymorphous teeth and cilia up to 4–5 cells long or more; cells near mouth *ca.* 20–25 μ. Capsule ovoid, its wall (4-) 5-stratose; outer layer of larger cells with nodular thickenings; their thickness equal to that of the inner layers; the inner cell layer with semiannular bands (partly incomplete or obsolete in middle of tangential wall). Elaters 6–7 μ in diameter; spores 12–15 μ, finely verruculose, yellowish-brown.[2]

Type

England.

Distribution

A very widespread species found throughout most of the tundra and taiga regions of the northern hemisphere, but absent in the southward extensions of taiga (and/or tundra) in the southern Appalachians and Cordilleras.

Found throughout northern Europe (northern Sweden, Norway, Finland, Russia), the Spitzbergen Islands, southward into England and Ireland, Denmark, the Faroes, Germany, southward to Spain in the Pyrenees and their periphery (6) and to Switzerland and northern Italy (12, 15). Also to Austria, Bucovina, and Bulgaria, and east to the USSR. Eastward extending into Asia (north and east Siberia, teste Arnell; Novaya Zemlya; Japan; Formosa (as *Lophozia asymmetrica* Horikawa) (7); Manchuria; China, in Yunan Prov.). Also on Jan Mayen Island and in Iceland (Hesselbo (8)).

In North America transcontinental, from Alaska and Yukon, southward to British Columbia, Alberta and eastward to the District of Keewatin and Manitoba. In eastern Canada and Greenland, as follows:

ELLESMERE ISLAND: Foot of Mt. Pullen, S. of Alert, NE. Ellesmere at 82° 25′ N. (*Schuster 35101, 35318a, 35340a, 35111, 35103a, 35121a, 35106,*

[2]The preceding diagnosis is so worded that the italicized characters separate the taxon from both *T. heterophylla* and the following two varieties of *T. quinquedentata.*

35314a, *35313*, *35102*, *35344*, *35101d*, *35134*, etc.; The Dean, 5–6 miles SE. of Alert, 82° 25′ N., 62° 05–15′ W. (*Schuster 35407a*, *35182b*, *35406b*, *35393b*, *35183a*, *35174*); E. edge of United States range, 9–10 miles W. of Mt. Olga, 82° 24′ N., 65° 10–30′ W. (*Schuster 35597*, *35579a*, *35593 p.p.* partly var. *grandiretis*); "Grant Land," Lat. 82° 27′ N. (as *Lophozia gracilis*, in NYBG; L. J. Wolf on Peary Polar Exped., July 1906!); "very common" in the Hayes Sound region, south Coast, and southwest Coast, with perianths at Goose Fiord (2).

E. GREENLAND: Cape Dalton; Fjord N. of Cape Dalton; Cape Daussy; Cape Ravn; also between 74° 30′ N. and 66° 58′ N.; Danmarks Ö, Heckla Havn, Gaaseland, Gaasefiord, Kobberpynt, Röde-Ö's Vestskraent, Runde-Fjeld, and Cape Stewart, all in Scoresby Sound; Hurry Inlet; Cape Parry; Kaiser Franz Josef Fjord; Röhss Fjord; Clavering Ö; Cape Mary; Lille Pendulum Ö (9, 10).

W. GREENLAND: Thule; Inglefield Gulf (77° 28′ N.); Godthaab; also "from Uvdle (76° 35′) to S. point." General on the Greenland coast, northward at least to Low Point (83° 06′ N.), Cape Benet (83° 02′ N.) and Lemming Fjord (82° 53′ N.), and probably general on ice-free land.

DEVON ISLAND: *fide* Steere (27).

DISTR. OF KEEWATIN: *fide* Steere (27).

BAFFIN ISLAND: Pond Inlet, on N. Baffin (Hesselbo), and Exeter Sound, in C. Baffin (Harmsen and Seidenfaden).

MELVILLE PENINSULA: Igloolik, Cape Elizabeth, Itividleriaq and Vansittart I. (Hesselbo).

LABRADOR: *fide* Macoun (11).

QUEBEC: Oka and Rigaud; Cap à l'Aigle; Sacré-Cœur, cté. de Rimouski; Rimouski; Ste. Anne de la Pocatière, etc. (Lepage); S. of Cairn I., Richmond Gulf; Manitounuck Sound; near Fishing Lake Creek, Richmond Gulf; Great Whale R.; all on east coast of Hudson Bay (21); Cape Aigle (*Macoun 3157*); Mt. Albert, Gaspé (O.D. Allen, 1881; as *J. incisa?!*).

NEWFOUNDLAND: Benoit Brook, W. Nfld.; Cow Head, Port au Choix, St. John I., St. Anthony, all in N. Peninsula; Hampden and Norris Arm, C. and NE. Nfld. (4).

NOVA SCOTIA: Margaree, Cape Breton I.; Aspy Bay (11).

NEW BRUNSWICK: Woodstock (11).

ONTARIO: Sudbury Jct.; Otter Head, Lake Superior; Lake Nipigon; Dawson Rte., W. of L. Superior (11).

Extending southward to the United States (Maine, New Hampshire, Vermont, Connecticut, New York, Michigan, Wisconsin, and Minnesota).

Ecology

Widespread almost wherever there are rock outcrops. The species is quite rare in the southern edge of the taiga, but extends to the northern edge of the tundra (83° 06′ N. Lat.). The distribution probably somewhat affected by a "weak affinity" for basic rocks (perhaps most frequent on basic intrusive

rocks, such as diabases and diorites, and other Ca and Mg rich basalts), with the consequence that the species is quite rare in most of the New England mountains, except where local outcrops of basic rocks occur.[3] Although often occurring on densely shaded cliffs, the species also occurs in open sunny sites.

"The species, in its main range, occurs under a great variety of conditions, as long as there is some integral connection between its substrate and rock exposure . . . essentially epipetric, but . . . only occasionally a pioneer species on rocks: it usually occurs on the crests of cliffs, over thin soil; from these it spreads into the crevices: reacting like a typical chasmophyte. From the crevices and cliff-crests it has limited ability to spread over exposed vertical rock faces, forming eventually dense mats whose size is limited by ice-action and their own weight when wet." (Schuster (23)). On rock outcrops, particularly in the Great Lakes area, are usually associated various species of the *Lophozia–Scapania* community complex, among them *Eremonotus minutus*, *Diplophyllum taxifolium* (under conditions at the xeric extreme for the *Tritomaria*), as well as *Lophozia ventricosa*, *Lepidozia reptans*, *Anastrophyllum saxicolus* and *A. michauxii*, *Lophozia kunzeana*, *L. attenuata*, *L. barbata*, and *L. hatcheri*, as well as *Scapania undulata*, *nemorosa*, *subalpina*, and sometimes *mucronata*. The "requirements" and tolerances of *T. quinquedentata* are extremely similar to those of *Lophozia barbata*, and these two robust species often occur together. The occurrences noted above are at the more acid extreme of the range of the species, ranging from pH 4.8–5.5. At the more nearly basic end of its range, on basaltic rock walls, the *T. quinquedentata* occurs with *Lophozia hatcheri*, *L. quadriloba*, and *Scapania mucronata*, as well as occasionally *T. scitula* (Keweenaw Co., Michigan).

In tundra, and around rock-pools (in the microclimatic "Tundra Zone" around much of Lake Superior), under subcalcareous conditions, the species may occur with *Scapania degenii*, *Odontoschisma macounii*, and even *Ptilidium ciliare*, together with *Selaginella selaginoides*, *Primula mistassinica*, *Euphrasia* sp., and *Scirpus caespitosus callosus*, at a pH of from 5.5 to 6.8. Under similarly subcalcareous conditions, on thin soil over basic rocks, it may occur with *Tritomaria scitula*, *Scapania mucronata*, *Blepharostoma trichophyllum*, etc. Although reported from old logs, I have never seen the species on pronouncedly humicolous sites.

In the high Arctic (NE. Ellesmere Island) on mossy, snow-fed slopes, in full sun, together with *Lophozia alpestris* var. *major*, *Lophozia heterocolpa*, *L. floerkei* var. *hyperborea*, *L. quadriloba*, *Eremonotus minutus*, *Scapania polaris*, *Tritomaria heterophylla*, *Cephaloziella arctica*, *Solenostoma schiffneri*, *Odontoschisma macounii*, and *Cryptocolea imbricata*, etc. The plant occurs here often as a compact sun form, with coarsely bulging trigones and yellowish cell

[3]Regarded by Reimers (18; p. 207) as a "Silikatmoos"; this is not correct, and even Reimers reports the species from humus lying over gypsum. Loeske states it is distributed only in granitic areas in the Harz Mts. Such a restricted distribution pattern is certainly not generally the case in North America. Steere (26) finds, with the author, that it is common in areas of basic rocks, reporting it from "moist trap rock ledges," with such calciphiles as *Scapania cuspiduligera* and *Lophozia gillmani*.

walls. The leaves are dense, imbricate, strongly concave, and somewhat antically secund, suberect to erect-spreading, giving the plant a very different facies from the lax-leaved phases with nearly horizontally patent leaves which are commoner in less arctic stations. The species occurs here on weakly calcareous talus.

Differentiation

Usually very easily recognized and hardly to be confused with any other species, especially when mature and typically developed. Much more robust than our other species of the genus, averaging fully twice the width. *T. quinquedentata* differs from the other species of *Tritomaria* not only in size, but in (*a*) the very rare occurrence of gemmae, and their pale yellow-brown color; (*b*) frequent development of sex organs and capsules; (*c*) the often explanate leaves, which have a diagnostic form. The leaves vary from fully as broad as long (the length measured to the apex of the ventral lobe, which is by far the largest) to, usually, conspicuously broader than long.

A strongly isolated species, without close relatives. The very broad leaves (wider than long on all but extreme, lax, small-leaved modifications), and their tendency to spread almost horizontally, nearly in one plane, together with the almost uniform absence of gemmae, give the species an aspect quite different from our other species of *Tritomaria*. *T. quinquedentata* averages almost or fully twice the size of our other species of the genus, and usually has a more prostrate growth-pattern than our four other species (although *T. scitula* may be scarcely more ascending).

T. quinquedentata often forms large, almost pure mats, which bear perianths or androecia in most cases, but almost never bear gemmae. In this respect the species differs greatly from the other taxa of the genus, in which there is always abundant gemmae production, and infrequent or rare development of sex organs. The gemmae in *T. quinquedentata* are yellowish or yellowish-brown, produced in such small numbers that they are perhaps most often overlooked. Those of our other species are produced in large, rust-red masses, forming a conspicuous identifying feature.

Owing to the robust size and generally somewhat explanate, scarcely canaliculate leaves, *T. quinquedentata* is apt to be mistaken, on superficial grounds, with *Lophozia barbata*. Indeed, it was at one time placed into the subgenus *Barbilophozia* with *L. barbata*, and was even considered to be a mere variety of *L. barbata* (16). However, the constantly trilobed and extremely asymmetric leaves, usually cuspidate at the apices of the lobes, at once set off *T. quinquedentata* from *L. barbata* (in which the leaves are nearly symmetrical and commonly 4-lobed). In *T. quinquedentata*, furthermore, the antical end of the leaf is essentially transversely inserted, while in *L. barbata* the antical end (like the rest of the insertion) is strongly oblique.

In the high Arctic, *T. quinquedentata* is at times difficult to separate from the broad-leaved *T. heterophylla*. The differentiation between these two plants is treated under *T. heterophylla*.

Variation

Generally a relatively stenotypic species, with therefore a constantly recognizable facies. However, weak forms occasionally are found with narrowly obtrapezoidal leaves which are closely similar to the leaves of *T. scitula* in appearance.

Southward usually with the plants somewhat prostrate, and with often quite patent, fluted leaves, only the ventral lobe generally being distinctly incurved. In close-leaved extremes, the leaves are more transversely oriented but do not become strongly concave. By contrast, in the far north the dominant phases of the species are strongly concave-leaved, the transversely oriented and often suberect, imbricate leaves being often almost hemispherical. Associated with this, they lack the distinctly fluted appearance so often marked in the more patent-leaved southern phases. These differences hardly warrant systematic recognition, being probably primarily environmental in origin.

By contrast, the following variants are clearly genetic in origin:

3. *Tritomaria quinquedentata* var. *grandiretis* Buch and Arnell, *in* Arnell, Svensk Bot. Tidskr. 44 (1), 84, 1950.

Similar to typical *T. quinquedentata*, but somewhat *paler and more pellucid* in appearance. *Cells markedly larger:* the marginal of the lobe apices 23–28 (30–35) μ; the subapical and lobar cells (21) 23–28 × 24–35 μ; the median cells (23) 25–32 × 30–42 μ; basal cells 23–32 (35) × 35–48 (56) μ.

Type

The first locality mentioned by Arnell, from Novaya Zemlya, 72° 20′ N. Lat. may be so considered.

Distribution

Known only from northern Siberia and Scandinavia (Sweden: Torne Lappmark at 68° 07′ N. Lat.) and as follows: ELLESMERE ISLAND: Moist, snow-fed scree slope, on humus, *ca.* 2000–2400 ft., east edge of the United States range, *ca.* 9–10 miles due west of Mt. Olga, *ca.* 82° 24′ N., 65° 20–30′ W. (*R. M. Schuster 35572a, 35593*). Occurring admixed with typical *T. quinquedentata*, *Eremonotus minutus* var. *grandis*, *Lophozia quadriloba* var. *grandiretis*, and *Scapania*.

The var. *grandiretis* has been considered to be (1) a putative polyploid derivative of normal *T. quinquedentata*. The two North American collections, in which the plants are admixed with typical *T. quinquedentata*, also suggest such an explanation, the intermingled plants of the two taxa showing the following differences in cell size:

	T. quinquedentata (typical)	var. *grandiretis*
Marginal cells of lobes	17–21 (23) μ	23–28 (30–35) μ
Subapical and median cells of lobes	16–21 (23) × 18–23 μ	(21) 23–28 (30) × 24–35 μ
Median cells	21–24 × 25–35 μ	(23) 25–32 × 30–42 μ
Basal cells	20–25 × 35–45 μ	23–32 (35) × 35–48 (56) μ
Oil-bodies, per subapical and median cell	3–7 (8–10)	9–17 (18–19)

Of these differences, that in the number of oil-bodies per cell is at once striking and systematically important.

4. Tritomaria quinquedentata var. turgida (Lindb.) Weim.

Jungermannia quinquedentata var. *turgida* Lindb., K. Vet. Ak. Handl. 23, 5, 1889.

Arctic form of *T. quinquedentata*, often with facies of *Saccobasis polita*, normally growing in bogs or wet polygon ground (with such species as *Calliergon sarmentosum*, *Drepanocladus revolvens* and *uncinatus*, *Oncophorus wahlbergii*). Usually brownish-tinged; leaf-lobes usually very short and broad, obtuse or virtually so, the two antical hardly cuspidate, the postical normally not at all cuspidate. Cells often slightly larger than in typical *T. quinquedentata*, with very coarse, bulging trigones (in this respect often closely approaching var. *grandiretis*, with which it may prove necessary to unite it).

Type

Jenissei R., Siberia.

Distribution

A high subarctic and arctic derivative of *T. quinquedentata*. Persson (17; pp. 284–285) discusses this plant, stating it is "rather common" in northern Scandinavia, "but seems to be absent from Central Europe." Müller, subsequently reports it from one station in the Tyrolese Alps (Spiegelkogel, 3400 meters).

In North America reported as follows:

E. GREENLAND: Hurry Inlet (9); also Cape Stewart, at Scoresby Sd., and Dove Bugt (76° 40′ N. lat.), *fide* Jensen.

ELLESMERE ISLAND: numerous localities in the southern half (2); see, however, the appended discussion.

ALASKA: widespread, from the E. Pacific Coast to Central Pacific Coast, the Alaska Range, the Aleutian Islands, and the Bering Sea district (St. Lawrence Island), according to Persson (17).

Persson (17) states that he has tried to determine "whether this variety might merit a higher systematic rank . . . the statements about a bigger size of the cells, which, combined with the northern distribution, suggested it possibly to be a substituting species with a higher number of chromosomes." He was unable to come to any definite conclusion, "the characters, especially the size of the cells, being too varying. Yet this form is of interest . . . because of its likeness to *Saccobasis*." In fact, Frye and Clark (5) erroneously reported *Saccobasis* from Thumb Bay, Alaska, the material proving to be *T. quinquedentata* var. *turgida* (17).

According to Persson (17) "this interesting variety is reported to be one of the most common hepatics brought home by the "Fram" expedition, for Arctic N. America (Bryhn, 1907) but I have not seen it again reported for N. America." The dubious status of this variant has led me to study the Ellesmere Island collections reported as var. *turgida* by Bryhn (2), with the following results: (1) The plant from Framshavn, lat. 78° 45′ N. (*Simmons 753*)

is *Lophozia hatcheri* Evs., mixed with traces of *Cephaloziella arctica* and *Anastrophyllum minutum*. (2) A second collection, same data (*Simmons 754*) is also *Lophozia hatcheri*, mixed with *Cephaloziella arctica* ("*C. divaricata*" on label). (3) A third collection, same data (*Simmons 739*) consists of pure *Lophozia hatcheri*, freely provided with brick-red gemmae. (4) A fourth collection, same data (*Simmons 740*) consists of *Lophozia hatcheri* mixed with *Cephaloziella arctica*. (5) A collection from Bedford Pim Island, off the east coast of Ellesmere Island, lat. 78° 40′ N., (*Simmons 1357*) labelled simply "*Lophozia lyoni*" (=*Tritomaria quinquedentata*) on the small (inner) packet, but "*Lophozia quinquedentata turgida*" on the outer packet, is a yellow-brown, thick-stemmed, small-leaved modification of typical *Tritomaria quinquedentata* (mod. *parvifolia–pachycaulis–colorata*). I cannot consider this plant to be varietally distinct from *T. quinquedentata*. Associated with the smaller leaf size, the leaves are more nearly symmetrically trilobate, somewhat narrower than usual. The lobes are typically all cuspidate with a mucro formed of 1–2 hyaline, enlarged cells. Occasionally the postical lobe is blunt, but this usually is associated with development of androecia, the perigonal bracts usually possessing blunt or rounded postical lobes. In both leaf-shape and size these plants closely approximate *Tritomaria heterophylla*; they differ from this at once in the smaller cell size (16–18 μ wide in the leaf-middle and at bases of leaf-lobes, only rarely and locally to 18–20 μ wide in the median cells; 15–18 μ on the lobe-margins), the absence of vineous pigmentation, and the total lack of gemmae. Associated evidently with the habitat, the plants have antically secund, saucer-like, concave leaves, and the leaves are suberect, thus giving the plants a facies much like that of *Lophozia binsteadii*. Admixed with these plants are traces of *Blepharostoma trichophyllum* var. *brevirete*, *Anthelia juratzkana*, *Cephaloziella arctica*, and *Odontoschisma macounii*. (6) A sixth collection, same data as No. 5, (*Simmons 1325*) labelled "*Lophozia lyoni turgida*," consists of several stems of *Tritomaria quinquedentata*, a phase with rather narrow, concave, suberect leaves. The leaf-lobes are relatively narrow and in general all sharply cuspidate. Hence these plants cannot be assigned to the var. *turgida*, unless that taxon be delimited very differently than by Persson (17). (7) Finally, a seventh collection, from Bedford Pim Island (*Simmons 1320*), supposedly "*Lophozia lyoni turgida*", consists of *Lophozia hatcheri*.

The preceding examination of seven Ellesmere Island collections assigned by Bryhn (2) to *Tritomaria quinquedentata* var. *turgida* (*Lophozia lyoni* var. *turgida*) thus demonstrates that Bryhn had no clear concept of this variant. Of the seven collections, five consisted of a yellow-brown, dense-leaved, concave-leaved phase of *Lophozia* (*Barbilophozia*) *hatcheri*, and two included an analogous phase of *Tritomaria quinquedentata*. The latter cannot, in my opinion, be separated as a distinct variety from *T. quinquedentata*, representing a mere xeromorphic, dense-leaved, mod. *parvifolia* with unusually concave and suberect leaves. Both collections have unusually narrowly triangular lobes, and possess almost constantly and uniformly mucronate lobe-apices.

As is well known, the small-leaved phases of *Tritomaria quinquedentata* "tend" to develop narrower than usual leaves, the leaf-shape undergoing heterogonic growth. The present plants appear to represent such a phase. I therefore cannot accept any of the Bryhn (2) reports of this variety from Ellesmere Island as authentic. Furthermore, none of the plants I collected on the north coast of Ellesmere Island in 1955 correspond to the var. *turgida*, as restricted by Persson (17) to plants with leaf lobes "shorter and broader, little or not cuspidate, often obtuse or nearly so, . . ." and with the leaf cells "often . . . somewhat bigger, with extremely large, bulging trigones."

Plants approaching the var. *turgida* occur as far south as the Hudson Bay region. I have seen sparse, somewhat brownish-yellow plants from Northwest Territory (Farmer Island, 58° 25′ N., 80° 48′ W., *A.R.A. Taylor 200, Aug. 24, 1944, p.m.p.*). in which the ventral lobes are blunt to obtuse, often actually rounded, but the two antical lobes slightly cuspidate. In these plants the marginal cells of the lobes average 20–23 μ, thus being somewhat intermediate in size between those of "normal" *T. quinquedentata* and those of var. *grandiretis*; the median leaf cells are also very strongly collenchymatous. The plants grew admixed with *Lophozia heterocolpa* var. *arctica*, *Cephaloziella arctica*, *Scapania arnellii*, *Lophozia hatcheri*, etc.

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